# THE CHEMISTRY AND BIOCHEMISTRY OF HYDROXYMETHYLPYRROLES

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A thesis

submitted in partial fulfilment

of the requirements for the degree

of

**Doctor of Philosophy in Chemistry**

in the

**University of Canterbury**

by

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University of Canterbury

1999

# WORK IN THIS THESIS HAS APPEARED IN THE FOLLOWING PUBLICATIONS

Andrew D. Abell, Brent K. Nabbs and Alan R. Battersby,

*J. Am. Chem. Soc.* **1998**, *120*, 1741.

Andrew D. Abell, J. Christopher Litten and Brent K. Nabbs

*J. Chem. Soc., Chem. Commun.* **1998**, 919.

Andrew D. Abell, Brent. K. Nabbs and Alan R. Battersby,

*J. Org. Chem.* **1998**, *63*, 8163.

Brent K. Nabbs and Andrew D. Abell,

*Bioorg. Med. Chem. Lett.* **1999**, *9*, 505.

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# ABSTRACT

This thesis examines the chemistry and biochemistry of hydroxymethylpyrroles of the type **1.9** (X = OH). Pyrrolic compounds play an important role in many areas of chemistry and biology. A large number of biomolecules which are essential for life are derived from simple pyrrole precursors, *eg* porphyrins and haemoglobin. In addition, pyrroles have been utilised as building blocks and key intermediates in synthesis.

Chapter One gives a general introduction to the biological, chemical and medical applications of pyrroles, such as in the biosynthesis of uroporphyrinogen III **1.20**, their use as intermediates in organic synthesis, and in the development of latent mechanism-based inhibitors of serine proteases.

Chapter Two describes a sequence, based on a Mitsunobu displacement at the hydroxymethyl position of the chiral deuterium-labelled *N*-substituted hydroxymethylpyrroles **2.26a-d** and **2.27a-d**, as a means by which to determine the deactivating abilities of various *N*-substituents on the pyrrole ring. In this reaction, an $S_N2$ mechanism was found to be favoured over reaction via an azafulvenium intermediate **2.20** by employing *N*-trifluoromethanesulfonyl as the deactivating group. Suppression of this azafulvenium reaction pathway was found to be less effective with other deactivating groups, and the order for deactivation was determined to be triflyl > mesyl > BOC ≈ acetyl.

Chapter Three describes the development of a short, convenient and versatile synthetic route to dipyrromethanes which involves coupling of an *N*-magnesio pyrrole salt (derived from either an oxygen acetal or a thioacetal of pyrrole-2-carboxaldehyde) with a ring-deactivated chloromethylpyrrole. This methodology was extended to the preparation of dipyrromethanes containing a deuterium-label at the interpyrrolic methylene position. An X-ray crystal structure of the *N*-tosyl chloromethylpyrrole **3.29**

showed that the aromaticity of the pyrrole ring was significantly reduced by the introduction of the electron withdrawing group onto the pyrrole nitrogen.

Chapter Four describes a $^1$H NMR investigation into the mechanism of hydrolysis of *N*-acylhydroxymethylpyrroles. In this study, the chiral deuterium-labelled *N*-(*N*-phthalyl-*L*-leucinyl)hydroxymethylpyrroles **4.8b** and **4.8c** in $CD_3CN$ were treated with potassium hydroxide in the presence of an equivalent of *S*-(+)-*sec*-butylamine. $^1$H NMR spectral analysis showed that the hydrolysis proceeds by an initial intramolecular *N*- to *O*-acyl transfer with retention of configuration at the labelled centre. Evidence for the subsequent release of an azafulvene **4.3** was gained from the observed scrambling of the deuterium-label at the *exo*-methylene position of **4.11** and **4.12** on trapping with an external nucleophile, either *S*-(+)-*sec*-butylamine or **4.11**, respectively.

Chapter Five describes the application of *N*-substituted hydroxymethylpyrroles as mechanism-based inhibitors of serine proteases. As a result, a number of alternate methodologies to incorporate the hydroxymethylpyrrole-moiety into an extended peptide-like sequence were attempted. From these synthetic approaches the pyrrole-based peptidomimetics **5.8a**, **5.8b**, **5.9a**, **5.9b** and **5.10b** were prepared and subsequently assayed for α-chymotrypsin inhibition activity. These compounds were found to be modest inhibitors of α-chymotrypsin, with none proving to be better inhibitors than previously discussed examples.

Chapter Six details the isolation and properties of some unexpected pyrrole-based molecules that were obtained from the attempted *N*-acylation of the 5-formylpyrrole-2-carboxamides **5.47a-c**. Attempts to *N*-acylate **5.47a-c** with hydrocinnamoyl chloride using the DMAP methodology gave the pyrrolizin-3-ones **6.1a-c**. These compounds were identified on the basis of one and two dimensional NMR spectral techniques. Conformation of the structure of the pyrrolizin-3-one **6.1a** was obtained by a single crystal X-ray analysis. Attempts to *N*-acylate **5.47a-c** with an acid chloride using the sodium hydride methodology gave the azafulvene dimers **6.6a-c**. Single crystal X-ray analysis of **6.6b** and **6.6c** revealed that each of these dimers were only a single

diastereomer, and were further dimerised by non-covalent interactions. Spectroscopic evidence is discussed which indicates that the non-covalent dimeric structure was also present in solution. We suggest that the diastereoselective synthesis of **6.6b** and **6.6c** was controlled by this ability to form the non-covalent dimers, and so represents an example of molecular self-assembly.

Chapter Seven describes a general synthesis of 5-acylpyrrole-2-carboxaldehydes which utilises a Stille coupling reaction between a stannylpyrrole and a (fatty) acid chloride. This methodology was used to prepare a series of 5-acyl-2-hydroxymethylpyrroles, including the previously reported natural product mycalazol 11 **7.11**. These compounds, together with a 5-carboxamido-2-hydroxymethylpyrrole, were assayed for *in vitro* cytotoxicity against the P388 cell line. From this assay we have found that an increased chain length leads to greater biological activity, and that an acyl side chain has greater activity relative to a carboxamido side chain.

# ABBREVIATIONS

| | |
|---|---|
| BOC | *tert*-butoxycarbonyl |
| BOC-ON® | 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile |
| camph | camphanate |
| Cbz | carbobenzyloxy |
| Δ | heat |
| 1,2-DCE | 1,2-dichloroethane |
| DCC | 1,3-dicyclohexylcarbodiimide |
| DEAD | diethyl azodicarboxylate |
| DMAP | 4-dimethylaminopyridine |
| DMF | *N*,*N*-dimethylformamide |
| DMSO | dimethylsulfoxide |
| EDCI | 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride |
| EWG | electron withdrawing group |
| HMB | hydroxymethylbilane |
| HOBt | 1-hydroxybenzotriazole |
| HRMS | high resolution mass spectrometry |
| Hünig's base | *N*,*N*-diisopropylethylamine |
| $IC_{50}$ | concentration required to inhibit cell growth by 50% |
| mesyl | methanesulfonyl |
| NMR | nuclear magnetic resonance |
| NOE | nuclear Overhauser enhancement |
| PBG | porphobilinogen |
| Pht | phthalyl |
| PPTS | pyridinium *para*-toluenesulfonate |
| rt | room temperature |
| SEM | 2-(trimethylsilyl)ethoxymethyl |
| THF | tetrahydrofuran |
| tosyl | *para*-toluenesulfonyl |
| triflyl | trifluoromethanesulfonyl |

# ACKNOWLEDGMENTS

Many thanks to my supervisor Dr Andrew Abell for his help and guidance throughout the course of this thesis.

I would like to thank the technical and academic staff in the Chemistry Department for their assistance. In particular, special thanks go to Bruce Clark for mass spectra, Rewi Thompson for infrared spectra, Gill Ellis for the P388 cytotoxicity assays, and Professor Ward Robinson for his help with X-ray crystallography.

I would also like to thank all the people who have helped me through the last 3 years with their friendship and support. Special thanks go to Andrew Phillips, Michael Stewart, Andrew Harvey, Barney May and to everyone in Lab 863 for their wisdom, patience and good humour.

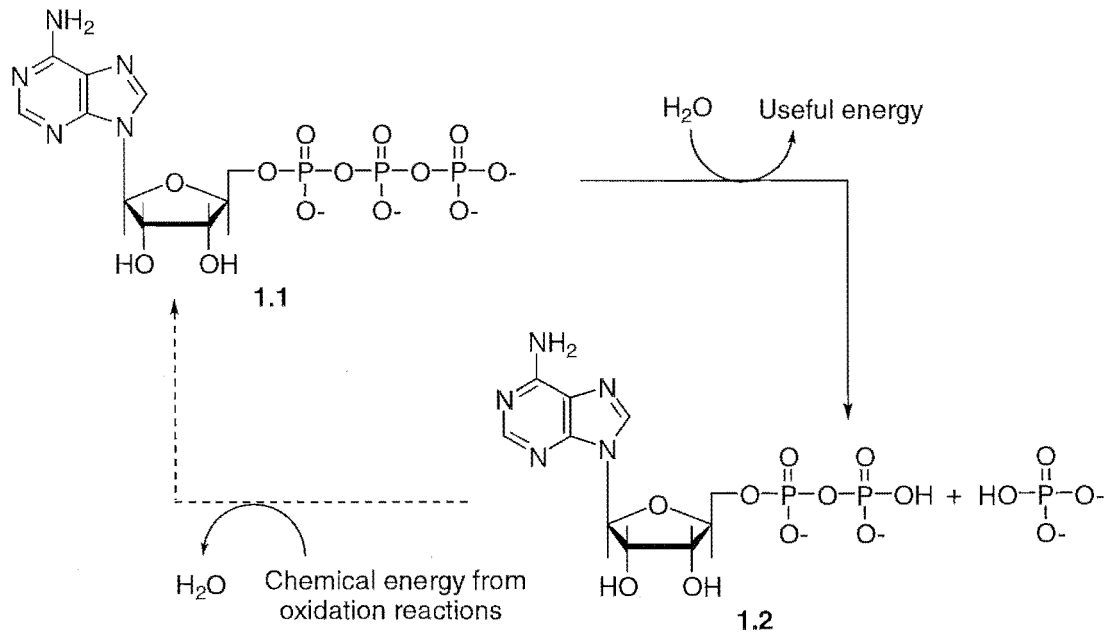
I would also like to thank my parents, Kay and Ken, for all their support, without which I would not have made it this far.

# CHAPTER ONE

# INTRODUCTION

## *1.1. Modulation of chemical reactivity*

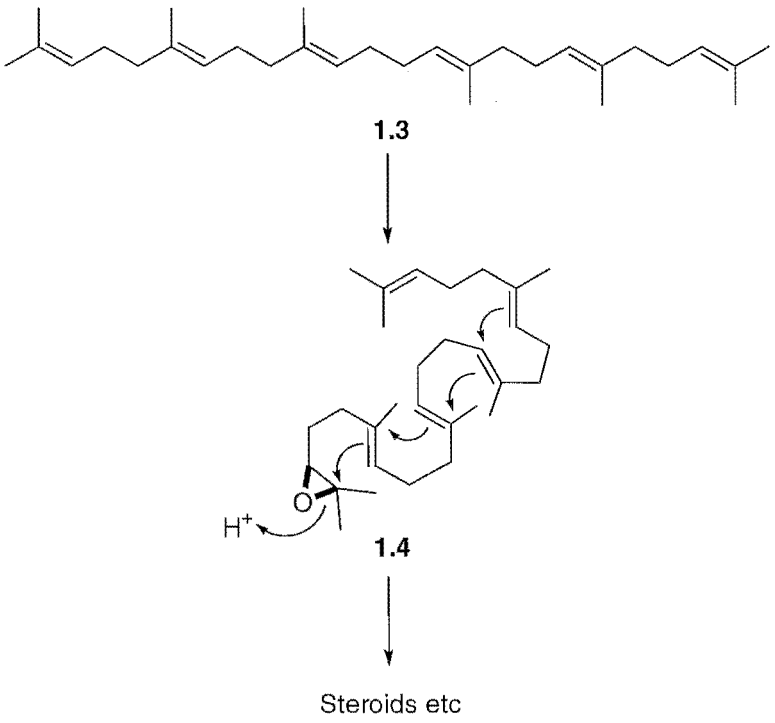
The ability to manipulate or modulate the reactivity of a molecule in a controlled manner is of fundamental importance in many areas of chemistry and biology. However, a fine balance exists in a molecule between chemical stability and useful chemical reactivity. If the molecule is too stable, it will not undergo the desired chemical transformation. On the other hand, if the molecule is too reactive its chemical reactivity will be unable to be controlled, resulting in the formation of undesired side products. In nature, this careful control of chemical reactivity is necessary in order to drive the vast multitude of chemical reactions which make up life. For example, the energy requirements for most biochemical processes are supplied by a substance called adenosine triphosphate (ATP) **1.1**. The 'energy' in ATP is stored in such a way that it can be released on demand to drive essential biochemical reactions that are energetically and thermodynamically disfavoured. This is achieved by coupling the disfavoured biochemical reaction with the energy releasing hydrolysis reaction of ATP to adenosine




**Scheme 1.1.** Hydrolysis and regeneration of ATP.

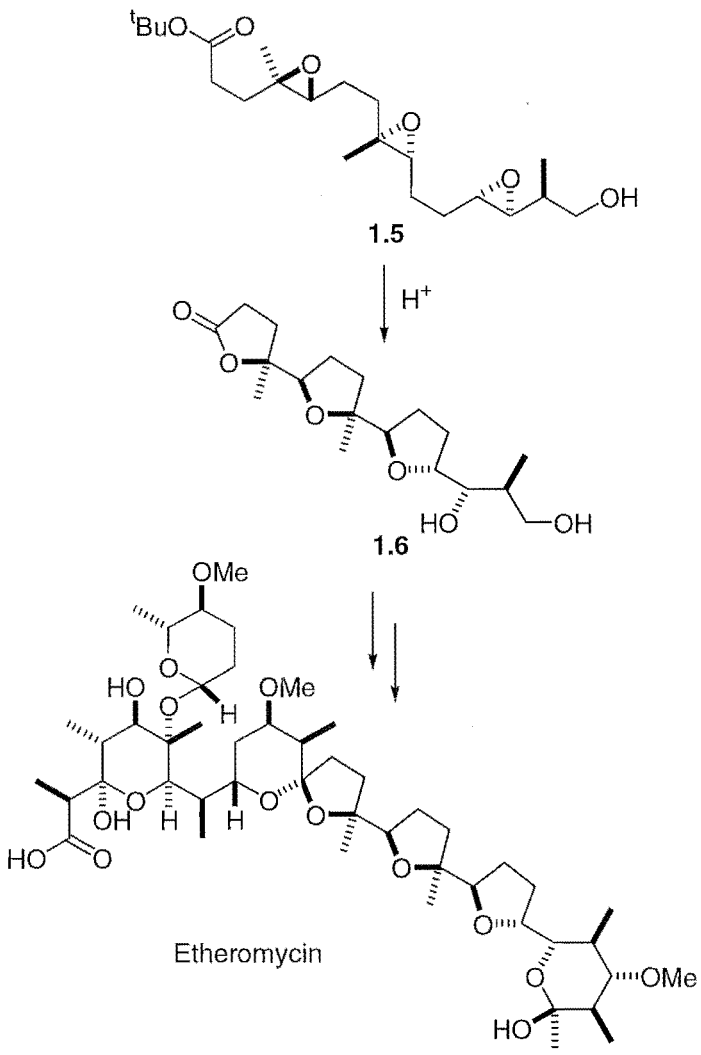
diphosphate (ADP) **1.2** and inorganic phosphate ($P_i$) (Scheme 1.1). The ADP formed can be subsequently converted back into ATP by coupling the energetically disfavoured phosphorylation reaction with an energetically favourable process, such as the oxidation of glucose.

Another example of controlled manipulation of chemical reactivity in nature is found in the biosynthesis of steroids. The ability of the key steroid precursor squalene **1.3** to undergo cyclisation is only activated after its regio- and stereospecific epoxidation to squalene monoepoxide **1.4** (Scheme 1.2).[1]



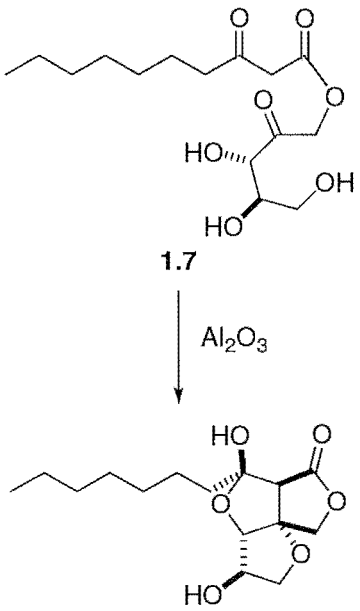

**Scheme 1.2.** Key cyclisation step in the biosynthesis of steroids.

The modulation of chemical reactivity has also been widely used in organic chemistry to control the outcome of a number of reactions. For example, in the biomimetic stererocontrolled synthesis of the etheromycin intermediate **1.6**, the latent ability of the precursor **1.5** to cyclise is only released upon its treatment with acid (Scheme 1.3).[2]




**Scheme 1.3.** Key cyclisation step in the biomimetic synthesis of etheromycin.

Another elegant illustration of controlled manipulation of chemical reactivity in organic chemistry can be found in the biomimetic synthesis of the microbial elicitor syringolide 2 **1.8**. In this example, exposure of the key *D*-xylulose derived intermediate **1.7** to basic alumina resulted in a facile triple cyclisation to give syringolide 2 **1.8** as a single stereoisomer (Scheme 1.4).[3]



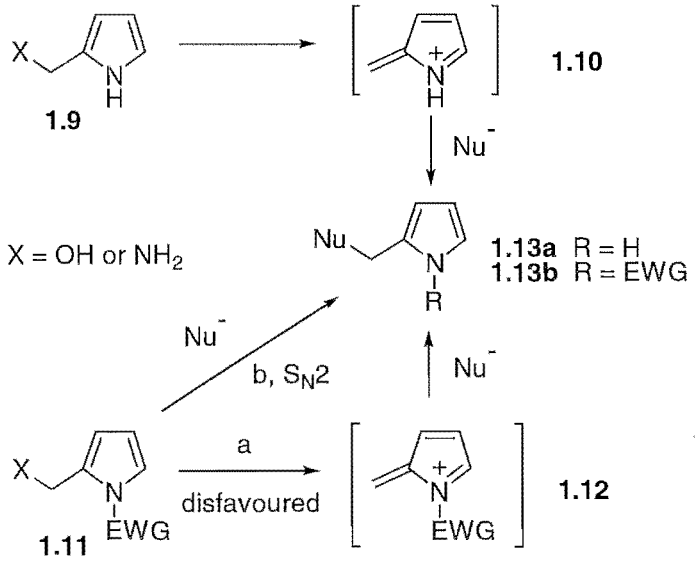

**Scheme 1.4.** Biomimetic synthesis of syringolide 2 **1.8**.

An important application of this controlled modulation of chemical reactivity of a molecule can be found in the area of medicinal chemistry. Here, the controlled release of latent (or masked) chemical reactivity of a molecule can be utilised, in a highly specific manner, so as to deliver a therapeutic effect to a specific site within a biological target.[4] For example, the inhibition of enzymes, a class of proteins which catalyse many of the important chemical reactions of life, forms the basis of many therapies for the treatment of a range of human metabolic diseases. However, the inherent chemical reactivity of many of the inhibitor compounds, combined with a lack of enzyme specificity, can lead to unwanted side reactions within the biological target, along with their associated side effects. One potential solution to this problem is to modify the inhibitor compound in such a way that its inherent chemical reactivity is masked, and is only released upon activation by the specific action of the target enzyme. This approach forms the basis of mechanism-based inhibitors, as discussed in further detail in Section 1.4.

## *1.2. Reactivity of 2-hydroxymethylpyrroles*

Pyrrolic compounds play an important role in many areas of chemistry and biology. A large number of biomolecules which are essential for life are derived from simple pyrrole precursors, *eg* porphyrins and haemoglobin. Likewise, pyrroles are key components of many biologically active natural products and biopharmaceuticals. In addition, pyrroles have also been utilised as building blocks and key intermediates in organic synthesis.

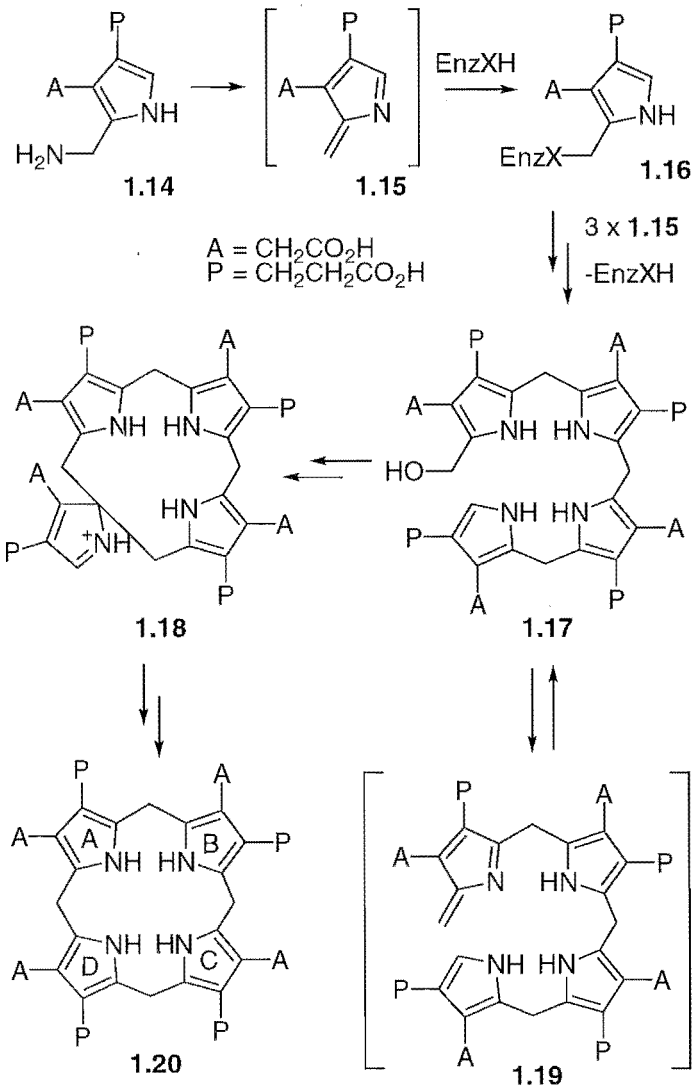
The introduction of an electron withdrawing group (EWG) onto the pyrrole nitrogen in compounds of the type **1.9** to give **1.11**, results in a decrease in the aromaticity of the pyrrole ring.[5] This substitution is thought to suppress the formation of the highly reactive azafulvenium species **1.12** (Scheme 1.5) due to the delocalisation of the nitrogen lone pair. Under these circumstances, ring-deactivated 2-substituted pyrroles of the type **1.11** react with nucleophiles via $S_N2$ chemistry to give products of the type **1.13b** (see Chapter 3 for specific examples). This is a further example of the modulation of chemical reactivity as discussed in Section 1.1. In this instance, the reactivity of **1.9** is




**Scheme 1.5.** Azafulvene formation of 2-substituted pyrroles.

modulated through the introduction of an EWG. In the absence of such deactivation, pyrroles of the type **1.9**, which have a suitable leaving group (X) at the 2-position, undergo loss of this leaving group to form the postulated azafulvene species **1.10**.[6] This electrophilic azafulvene species readily reacts with nucleophiles to give products of the type **1.13a** (Scheme 1.5).[5a,7]

Such a sequence involving azafulvenes is thought to be involved in the biosynthesis of hydroxymethylbilane (HMB) **1.17** and uroporphyrinogen III (uro'gen III) **1.20**, important intermediates in the biosynthesis of vitamin $B_{12}$ and related pigments




**Scheme 1.6.** Biosynthesis of uroporphyrinogen III **1.20**.

(Scheme 1.6).[8] In the biosynthesis of uro'gen III, four molecules of porphobilinogen (PBG) **1.14** are assembled together under the combined action of the enzymes hydroxymethylbilane synthase (PBG deaminase) and uroporphyrinogen III synthase (uro'gen III cosynthetase). In the first step of the biosynthesis, PBG gives rise to the azafulvene **1.15** which becomes covalently bound to the PBG deaminase enzyme via a dipyrromethane cofactor. This sets up the enzyme-substrate complex **1.16**, and three more molecules of PBG condense sequentially to **1.16**, again via the azafulvene **1.15**. This generates an enzyme bound tetrapyrrole, which is subsequently released as HMB **1.17**. The current view is that the azafulvene **1.19** is the intermediate which reacts with water to afford **1.17**.[9] Uro'gen III cosynthetase then acts on the HMB **1.17** to bring about cyclisation with ring-D inversion to yield uro'gen III **1.20**. Again, the mechanism of this cyclisation is thought to involve the azafulvene **1.19**. The exact mechanism of formation of the ring-D inverted uro'gen III **1.20** has been the topic of extensive speculation, and at least 25 biosynthetic schemes have been postulated.[8b] However, a number of isotopic labelling experiments have been carried out using both $^{13}C$- and $^{14}C$-labelled PBG,[10] and also with doubly $^{13}C$-labelled PBG,[8a,11] and these labelling experiments suggest that the *spiro*-compound **1.18** is an intermediate in the mechanism of this ring cyclisation.

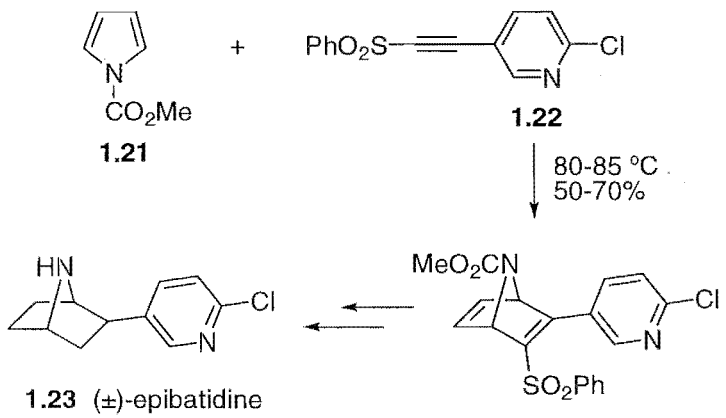
For pyrroles of the type **1.11** to be useful in organic and biological chemistry, the inherent reactivity of these compounds (*ie* azafulvene formation) must be able to be released in a controlled manner when, and only when, it is desired. This is able to be achieved through either the chemical or enzymatic removal of the electron withdrawing group from **1.11** to give pyrroles of the type **1.9** – which can then undergo azafulvene formation (see Scheme 1.5). This ability to selectively release the latent reactivity of pyrroles of the type **1.11** when desired, forms the basis by which these class of compounds are able to be utilised as useful synthetic intermediates and as mechanism-based inhibitors of enzymes. These applications are discussed in further detail in Sections 1.3 and 1.4.

## 1.3. Deactivated pyrroles as intermediates in organic synthesis

A wide variety of electron withdrawing groups have been employed on the pyrrole nitrogen in order to deactivate the ring system, and so allow its use as an intermediate in organic synthesis. Examples of the groups which have been employed to effect this ring-deactivation include methanesulfonyl,[12] phenylsulfonyl,[13] *tert*-butylcarbamoyl[14] and alkoxycarbonyls such as *tert*-butoxycarbonyl (BOC)[15] and ethoxycarbonyl.[16] Consequently, a large number of synthetic applications for ring-deactivated pyrroles have been described in the literature. What follows are some examples of the chemistry of pyrrole which is possible due to this ring-deactivation.

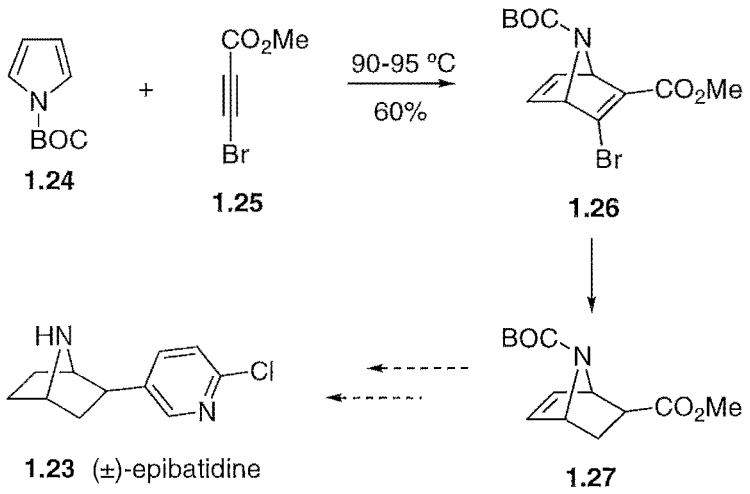
### Diels-Alder chemistry

Non-deactivated pyrroles and their derivatives tend to be unreactive towards Diels-Alder reactions.[17] In contrast, pyrroles which are substituted with a suitable electron withdrawing group on nitrogen are able to act as the diene component in Diels-Alder reactions.[18] For example, in the reported synthesis of (±)-epibatidine **1.23**, a potent analgesic alkaloid possessing a 7-azanorbornane structure, the key step involved a Diels-Alder reaction between the *N*-methoxycarbonylpyrrole **1.21** and a functionalised phenylsulfonyl acetylene **1.22** (Scheme 1.7).[19]




**Scheme 1.7.** Diels-Alder route to the synthesis of (±)-epibatidine **1.23**.

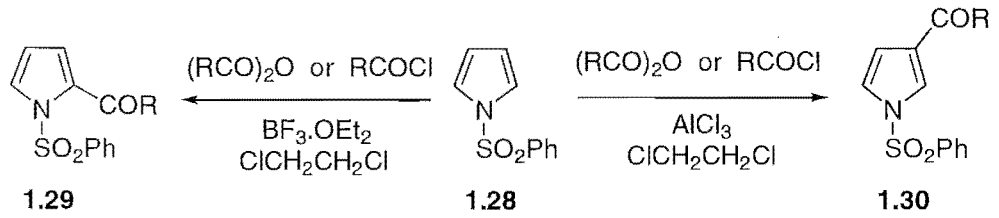
A further extension of this work has recently been reported by Singh and Basmadjian.[20] In this recent example, a Diels-Alder reaction between the *N-tert*-butoxycarbonylpyrrole **1.24** and the bromoacetylene **1.25** was used to generate the cycloadduct **1.26**. Hydrodehalogenation of **1.26** then afforded the desired 7-azabicyclo[2.2.1]heptene **1.27** – a possible intermediate in the synthesis of epibatidine **1.23** and related analogues (Scheme 1.8).




**Scheme 1.8.** Alternate Diels-Alder route to the 7-azabicyclo[2.2.1]heptane skeleton.

**Regiocontrolled acylation**

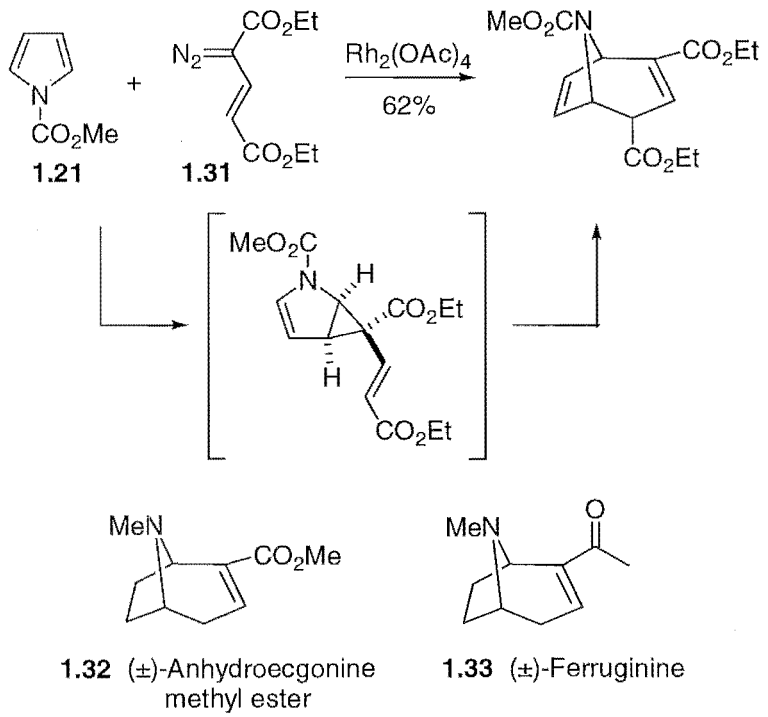
The regioselective acylation of deactivated pyrrole derivatives has been reported in a number of instances.[21] Pyrroles normally undergo Friedel-Crafts acylation at the C-2 position. However, when the pyrrole is *N*-substituted with a phenylsulfonyl group, as in **1.28**, the site of substitution on the pyrrole ring can be controlled by the Lewis acid catalyst employed. In the presence of boron trifluoride etherate acylation gives predominantly the 2-acyl derivative **1.29**. In contrast, in the presence of aluminium trichloride acylation occurs exclusively at the C-3 position to give **1.30** (Scheme 1.9).




**Scheme 1.9.** Regiocontrolled acylation of *N*-phenylsulfonylpyrrole **1.28**.

### Synthesis of the tropane skeleton

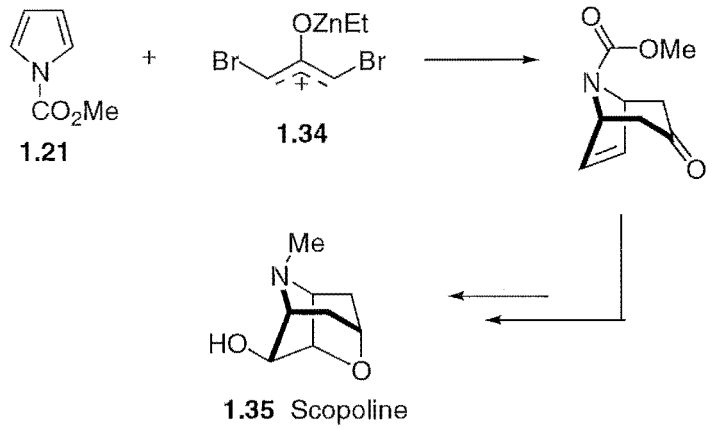
Deactivated pyrroles have been utilised in the synthesis of tropane alkaloids – an important class of natural products with extensive biological properties.[22] Consequently, a number of alternate methodologies have been employed to enable the preparation of compounds of this class. For example, Davies *et al.* utilised a rhodium-catalysed cyclopropanation/sigmatropic (Cope) rearrangement of the *N*-methoxycarbonylpyrrole **1.21** with a vinyldiazoester **1.31** to give access to the tropane skeleton (Scheme 1.10).[23]




**Scheme 1.10.** Synthesis of tropane alkaloids using ring-deactivated pyrroles.

A similar reaction with non-deactivated *N*-alkylpyrroles resulted in the formation of alkylation products.[23] This above methodology has been further extended by Davies *et al.* to enable the syntheses of the tropane natural products, (±)-anhydroecgonine methyl ester **1.32** and (±)-ferruginine **1.33** (see Scheme 1.10).[24]

A ring-deactivated pyrrole has also been used to prepare the tropane alkaloid scopoline **1.35**.[25] In this instance, the key step in the synthesis was a [4+3]-cycloaddition reaction between the *N*-methoxycarbonylpyrrole **1.21** and the oxyallyl carbocation **1.34** (Scheme 1.11).



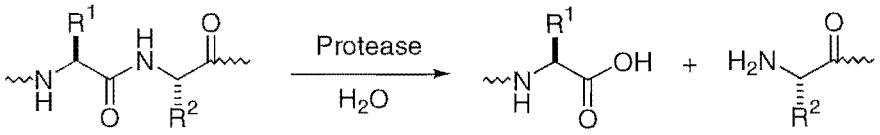

**Scheme 1.11.** Synthesis of scopoline **1.35** using a ring-deactivated pyrrole.

The use of electron withdrawing groups on the pyrrole nitrogen have also been employed for numerous other applications in organic synthesis. For example, ring-deactivated pyrroles have been observed to stabilise intermediates in photocyclisation reactions,[26] are readily reduced under Birch conditions,[27] allow the synthesis of 2-substituted pyrroles via α-lithiation,[28] and have been utilised in Wittig reactions.[26,29]

A number of non-deactivating groups have also been employed to protect the nitrogen and/or direct reaction of the pyrrole nucleus. These groups include methyl,[30] benzyl,[31] 2-(trimethylsilyl)ethoxymethyl (SEM),[32] trialkylsilyl[33] and dimethylamino.[34]

## 1.4. Hydroxymethylpyrroles as mechanism-based inhibitors of serine proteases

Proteolytic enzymes, or simply proteases, are an important class of enzyme within biological systems. These enzymes are characterised by their particular function, which is to catalyse the hydrolytic cleavage of peptide (or amide) bonds in proteins and polypeptides (Scheme 1.12).




**Scheme 1.12.** Protease catalysed hydrolytic cleavage of a peptide bond.

Proteases are divided into the four subclasses, serine, aspartic, cysteine and metallo-proteases, on the basis of the most important residue or functional group associated with enzyme activity. Each subclass of protease has a number of key functions, for instance digestion, blood coagulation, and hormone metabolism, and as such play an important role in the overall regulation and maintenance of normal biochemical processes.[35] An imbalance in the normal functioning of any of these enzymes can therefore lead to metabolic disorders and disease. Table 1.1 summarises representative examples of these proteases in their respective subclasses, along with a brief mention of their normal metabolic function.

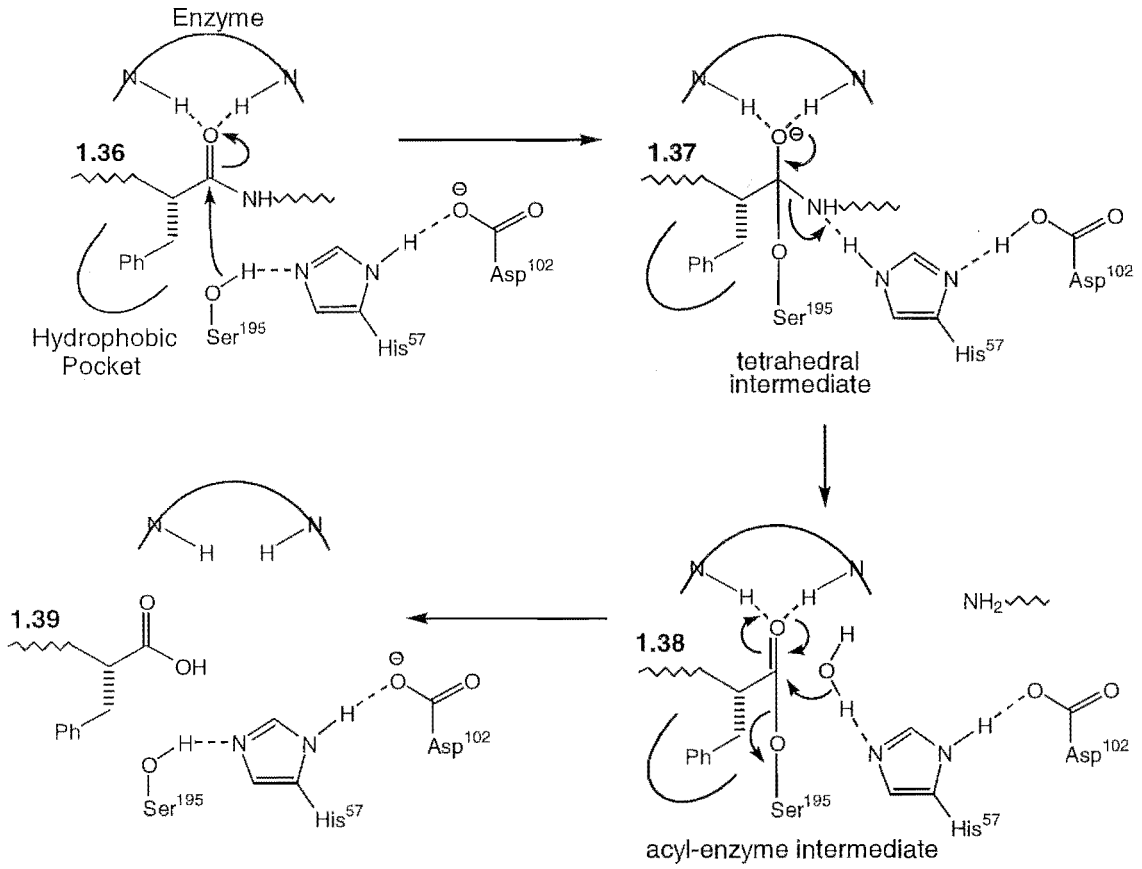
The following section of this introduction will deal specifically with the serine protease subclass of proteolytic enzymes, as this class of enzyme is the topic of some of the work detailed in this thesis (see Chapter 5 for work in this area).

| Protease subclass | Representative examples | Normal metabolic function |
|---|---|---|
| Serine | α-chymotrypsin, trypsin | digestion |
| | thrombin, plasma kallikrein | blood coagulation |
| | post-protein cleaving enzyme | hormone metabolism |
| | elastase, cathepsin G, tryptases | phagocytosis |
| Aspartic | renin | blood pressure regulation |
| | HIV protease | HIV replication |
| | pepsin, thermolysin | digestion |
| Cysteine | cathepsins B, H, L, calcium activated neutral proteases | protein turnover, bone resorption |
| | papain | digestion |
| Metallo | angiotensin converting enzyme | blood pressure regulation |
| | enkephalinases | analgesic function |
| | carboxypeptidase | digestion |
| | collagenase | tissue remodelling |

**Table 1.1.** Examples of proteases and their normal metabolic function.

The active site of a serine protease comprises a highly conserved catalytic triad of amino acids, for instance $Asp^{102}$, $His^{57}$ and $Ser^{195}$ as in α-chymotrypsin (the numbers 102, 57 and 195 denote the position of the key residues aspartic acid, histidine and serine, respectively, in the polypeptide chain).[36] The aspartic acid and histidine residues act as an electron source and sink, respectively, in a charge-transfer system that serves to increase the nucleophilic nature of the serine residue.[37] The proposed mechanism for the hydrolysis of a peptide bond by α-chymotrypsin is detailed in Scheme 1.13. The activated serine nucleophile attacks the peptide substrate **1.36** to form the tetrahedral intermediate **1.37**. The oxyanion of **1.37** is stabilised by hydrogen bonding to NH groups in an area of the enzyme backbone referred to as the 'oxyanion hole'. This tetrahedral

intermediate **1.37** is then converted into an acyl-enzyme intermediate **1.38**, with the release of a new *N*-terminal peptide. The acyl-enzyme intermediate **1.38** is finally hydrolysed by a molecule of water in the enzyme active site, which releases the *C*-terminal peptide **1.39** and regenerates the catalytic triad.[36]

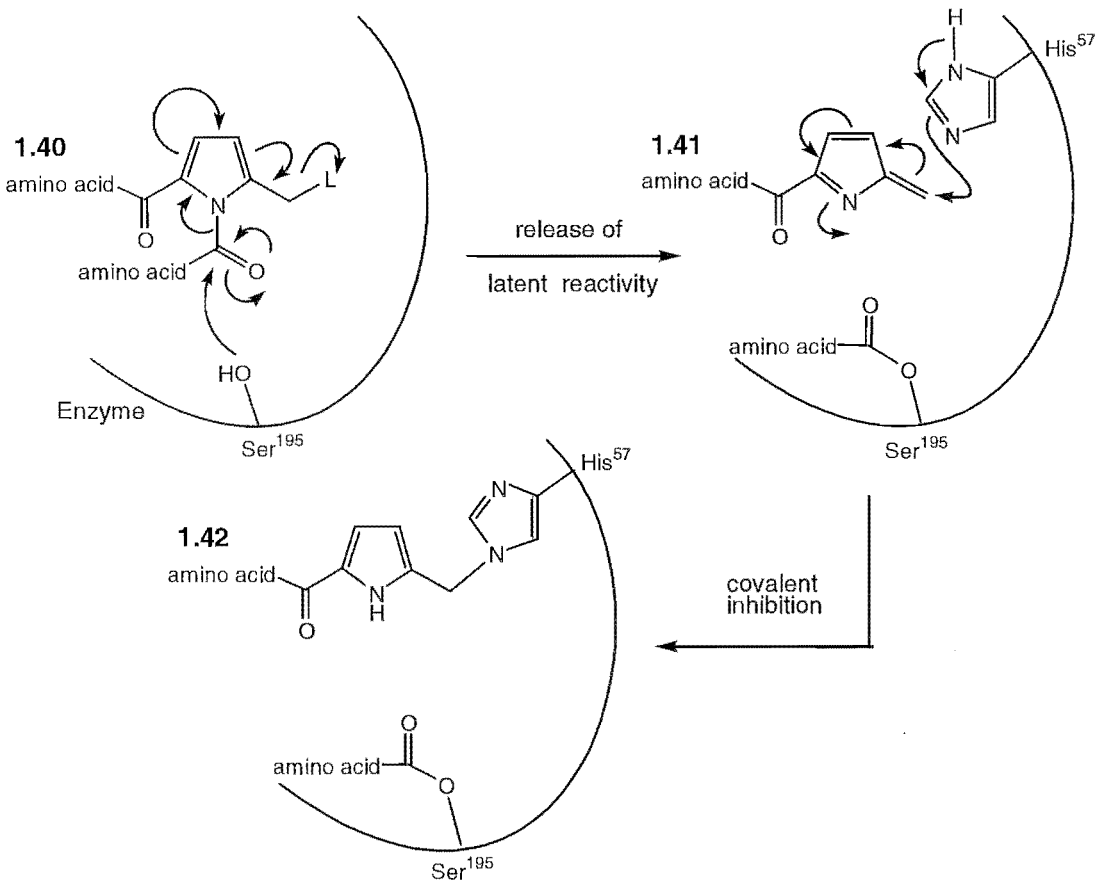



**Scheme 1.13.** Serine protease catalysed hydrolysis of a peptide bond.

An imbalance in the activity of serine proteases has been found to be involved in many pathological conditions. For example, enzymes such as α-chymotrypsin, human neutrophil elastase, cathepsin G and pancreatic elastase are thought to play key roles in the initiation and progression of number of human diseases such as digestive disorders, pulmonary emphysema, rheumatoid arthritis and pancreatitis, respectively.[38] Therefore,

the selective inhibition of these enzymes is a therapeutic goal for the treatment of these, and other related, conditions.

In view of this fact, we have developed *N*-acylhydroxymethylpyrroles as a new class of mechanism-based inhibitor of serine proteases. A mechanism-based inhibitor is a chemically non-reactive compound which is sufficiently similar in structure to the natural substrate to be bound in the active site of the target enzyme. Once in the active site, normal enzyme action produces a reactive species, which can result in the specific, efficient and irreversible inhibition of the target enzyme. In this case, the *N*-acylhydroxymethylpyrrole **1.40** (Scheme 1.14) is designed to mimic the enzyme's natural substrate (see Figure 1.1). Accommodation of this inhibitor **1.40** in the enzyme active




**Scheme 1.14.** Proposed mechanism of inhibition of *N*-acylhydroxymethylpyrroles **1.40**.

site would lead to the catalytic hydrolysis of the pyrrole *N*-acyl bond. The subsequent loss of the leaving group (L) from the 2-position of this non-deactivated pyrrole would produce the electrophilic azafulvene **1.41**, thereby releasing the latent reactivity of this class of mechanism-based inhibitor. The azafulvene thus formed could then be trapped by one of several suitable nucleophilic residues (*eg* His[57] as shown in Scheme 1.14) in the enzyme active site to give a bound pyrrole derivative **1.42** and an inhibited enzyme.

The enzyme's primary specificity pocket ($S_1$) is of main importance in determining which amino acids of the substrate (or inhibitor) can be accepted into the enzyme active site.[36] Schechter and Berger[39] devised terminology to describe the interaction between an enzyme and substrate on binding (see Figure 1.1). Numbering from the carbonyl end of the scissile amide bond (*ie* the peptide bond to be cleaved) to the amino terminal, the substrate (or inhibitor) amino acid residues are termed $P_1$, $P_2$, etc, while the corresponding subsites of the enzyme are termed $S_1$, $S_2$, etc. The substrate (or

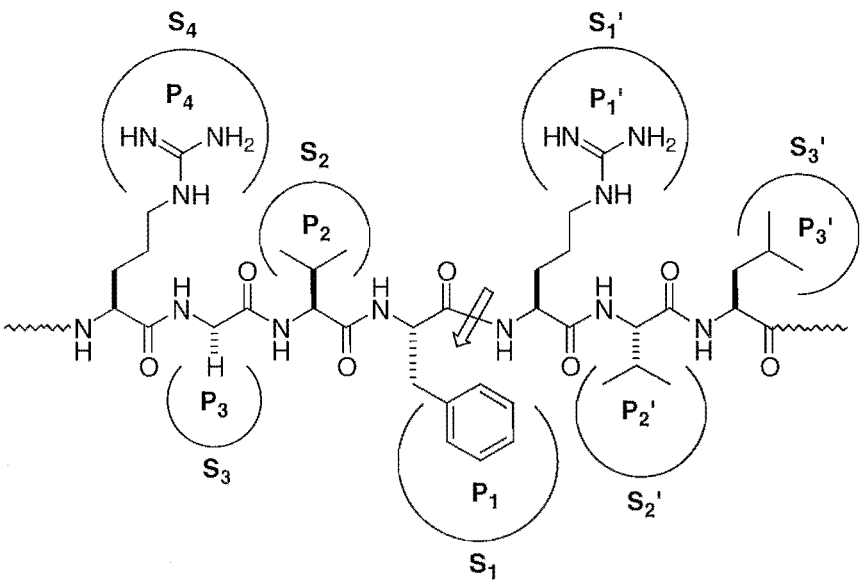



**Figure 1.1.** A section of a natural substrate of a serine protease such as α-chymotrypsin showing the interactions between the amino acid side-chains of the substrate ($P_n$, $P_n'$) and the corresponding subsites of the enzyme's active site ($S_n$, $S_n'$). The scissile amide bond is shown by an arrow.

inhibitor) residues going in the direction of the carboxy terminal from the scissile bond are termed $P_1'$, $P_2'$, etc, while the corresponding enzyme subsites are termed $S_1'$, $S_2'$, etc (see Figure 1.1).

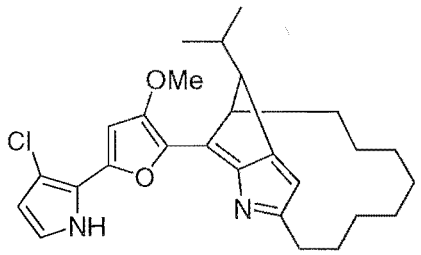
In the case of α-chymotrypsin, the $S_1$ subsite is a large well defined hydrophobic pocket and is known to accept aromatic and aliphatic residues positioned at the primary amino acid binding site ($P_1$). However, enzyme-substrate recognition is not based entirely on the interaction between the enzyme's primary specificity pocket ($S_1$) and the substrate's primary binding amino acid ($P_1$), but it is in fact influenced by neighbouring and longer range secondary interactions. For example, through serine protease inhibition studies it has been found that the residues $P_4$, $P_2$, $P_2'$ and $P_3'$, which are close to the scissile bond, are important for the recognition and binding of the substrate, as well as residues as far away from the active site as $P_{14}'$.[40] Therefore, by incorporating the hydroxymethylpyrrole-moiety into an extended peptide-like sequence we aim to generate better inhibitors through the potential for improved enzyme recognition and binding (see Chapter 5 for work in this area). The structure and relative position of the substrate binding sites of serine proteases has only become available through X-ray crystallographic studies,[41] NMR spectroscopy,[41a,42] and enzyme inhibition studies.[43]

## *1.5. Hydroxymethylpyrroles and azafulvenes in natural products*

An appreciable number of hydroxymethylpyrrole- and azafulvene-based natural products, exhibiting a wide spectrum of biological activity, have been isolated from a variety of natural sources. A brief selection of some of these compounds, along with their biological activity is presented in the following section.

### Roseophilin

In 1992, the azafulvene-based compound roseophilin **1.43** was isolated by Hayakawa *et al.* from the culture broth of an actinomycete identified as *Streptomyces griseoviridis* (Figure 1.2).[44] This compound showed significant cytotoxicity against the K562 human erythroid leukemia and KB human epidermoid carcinoma cell lines ($IC_{50}$ of 0.34 μM and 0.88 μM, respectively). The first total synthesis of roseophilin **1.43**, by a convergent and highly modular approach, has recently been reported by Fürstner and Weintritt.[45]




**1.43**

**Figure 1.2.** Roseophilin **1.43**.

### Mycalazols 1 – 12 and mycalazals 1 – 2

Recently, a series of fourteen pyrrole-based metabolites were isolated from a north-eastern Atlantic sponge *Mycale micracanthoxea*.[46] The authors of this work characterised twelve 5-acyl-2-hydroxymethylpyrroles (mycalazols 1 – 12) and two 5-

alkylpyrrole-2-carboxaldehydes (mycalazals 1 – 2) that differed in the length and degree of unsaturation of their side chain (see Figure 1.3 for representative examples). These compounds were assayed for cytotoxicity against the P388 murine leukemia, SCHABEL mice lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma and MEL28 human melanoma cell lines. In each case, these pyrrole-based metabolites were shown to possess moderate *in vitro* cytotoxicity ($ED_{50}$ of 1 – 10 μg $mL^{-1}$).

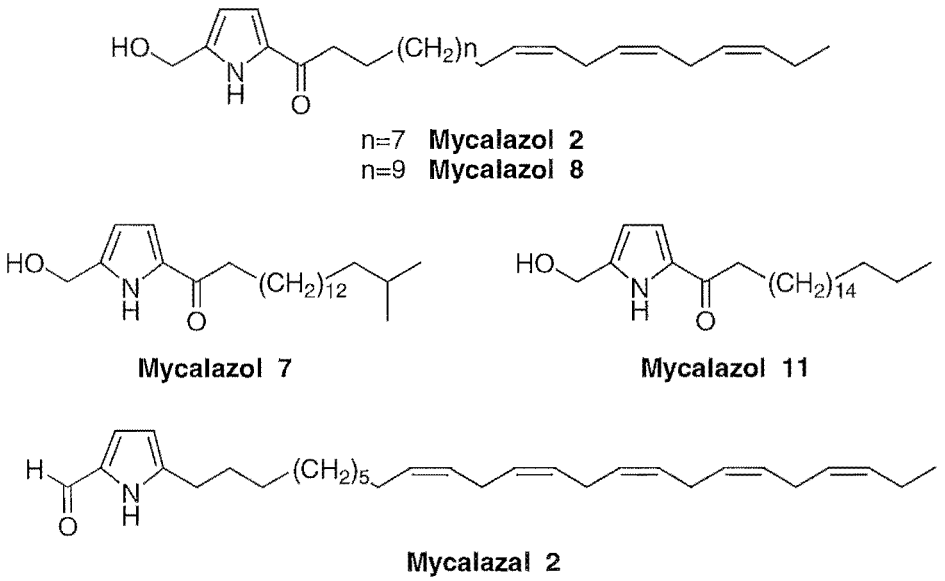
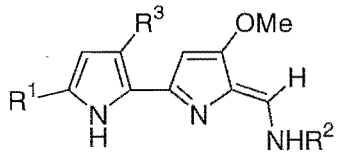



**Figure 1.3.** Representative examples of mycalazols and mycalazals.

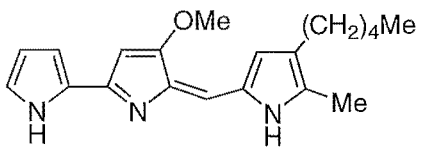
### Tambjamine and related alkaloids

A large number of azafulvene-based bipyrrolic compounds, collectively known as the tambjamine class of alkaloids **1.44a-j** (Figure 1.4), have been isolated from a wide range of marine organisms, including bryozoans, nudibranchs and ascidians.[47] The tambjamine alkaloids are reported to play a role in the chemical defence mechanism of the respective organisms, having been ascribed feeding and ichthyodeterrent properties.[47b] In addition, these compounds have also been found to show antimicrobial activity (5 – 50 μg $disk^{-1}$) against a range of bacteria, and inhibit cell division in the fertilised sea urchin egg assay at a concentration of 1 μg $mL^{-1}$ in seawater.[47a] A series of

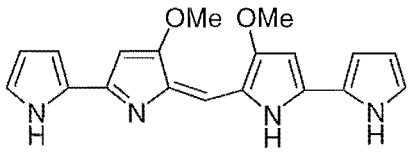
closely related linear tri- and tetrapyrroles have also been isolated from a variety of natural sources. For example, prodigiosin **1.45** has been isolated from both terrestrial and marine bacteria,[48] while **1.46** has been isolated from bryozoans, an ascidian and a mutant strain of the bacterium *Serratia marcescens*.[47b,49]




| | $R^1$ | $R^2$ | $R^3$ | Tambjamine |
|---|---|---|---|---|
| **1.44a** | H | H | H | A |
| **b** | Br | H | H | B |
| **c** | H | $CH_2CHMe_2$ | H | C |
| **d** | H | $CH_2CHMe_2$ | Br | D |
| **e** | H | $CH_2Me$ | Br | E |
| **f** | H | $CH_2CH_2Ph$ | H | F |
| **g** | Br | $CH_2Me$ | H | G |
| **h** | Br | $CH_2CH_2Ph$ | H | H |
| **i** | Br | $CH_2CHMe_2$ | H | I |
| **j** | Br | $CH(Me)CH_2Me_2$ | H | J |




**1.45**




**1.46**

**Figure 1.4.** Tambjamines A – J (**1.44a-j**) and related metabolites.

## *1.6. Work described in this thesis*

Chapter Two describes an investigation to quantify the deactivating abilities of various *N*-substituents on the pyrrole ring. It has previously been assumed that the introduction of an electron withdrawing group onto a pyrrole nitrogen, as in **1.11**, suppresses the formation of highly reactive azafulvenium intermediates **1.12** (see Scheme 1.5), but little direct evidence for this has been presented.

Chapter Three describes an application of this pyrrole ring-deactivation. In this work, a short, convenient and versatile route to dipyrromethanes was developed. This methodology was extended to the preparation of dipyrromethanes containing a deuterium-label at the interpyrrolic methylene position. These compounds could potentially act as probes for studying some of the key stereochemical issues associated with the biosynthesis of porphyrins such as vitamin $B_{12}$ and related pigments.

Chapter Four describes an investigation into the removal of the nitrogen electron withdrawing group from hydroxymethylpyrroles, thereby allowing the release of the latent reactivity of this class of compound. This investigation was primarily concerned with the mechanism by which this removal of the EWG proceeds.

Chapter Five describes the various synthetic attempts that were made at incorporating the hydroxymethylpyrrole functionality into an extended peptide-like sequence. This was attempted in order to develop mechanism-based inhibitors of serine proteases which were more closely based on the natural substrate, thereby increasing the enzyme specificity and selectivity for the inhibitor molecule. The isolation of unusual and interesting pyrrole-based molecules from some of these synthetic approaches is described in Chapter Six. The ability of a class of these pyrrole-based molecules to undergo possible molecular self-assembly is also discussed.

Finally, Chapter Seven describes the synthesis and P388 cytoxicity assaying of a series of analogues of the mycalazol natural products.

## 1.7. References for chapter one


1 Mann, J. *Secondary Metabolism.* Oxford University Press: Oxford, 1986, 2nd Edn, pp138-143.

2 Paterson, I.; Boddy, I.; Mason, I. *Tetrahedron Lett.* **1987**, *28*, 5205.

3 Henschke, J. P.; Rickards, R. W. *Tetrahedron Lett.* **1996**, *37*, 3557.

4 For an example see: Friedlos, F.; Denny, W. A.; Palmer, B. D.; Springer, C. J. *J. Med. Chem.* **1997**, *40*, 1270, and references therein.

5 (a) Jones, A. R.; Bean, G. P. Eds. *The Chemistry of Pyrroles.* Academic Press: London, 1977; (b) Katritzky, A. R.; Rees, C. W. Eds. *Comprehensive Heterocyclic Chemistry.* Pergamon Press: Oxford, 1984, Vol 4.

6 (a) Schauder, J. –R.; Jendrezejewski, S.; Abell, A.; Hart, G. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1987**, 436. (b) Miller, A. D.; Leeper, F. J.; Battersby, A. R. *J. Chem. Soc., Perkin Trans. 1.* **1989**, 1943.

7 Barcock, R. A.; Moorcroft, N. A.; Storr, R. C. *Tetrahedron Lett.* **1993**, *34*, 1187.

8 (a) Battersby, A. R.; Fookes, C. J. R.; Gustafson-Potter, K. E.; McDonald, E.; Matcham, G. W. T. *J. Chem. Soc., Perkin Trans. I.* **1982**, 2427; (b) Battersby, A. R.; Leeper, F. J. *Chem. Rev.* **1990**, *90*, 1261; (c) Blanche, F.; Cameron, B.; Crouzet, J.; Debussche, L.; Thibaut, D.; Vuilhorgne, M.; Leeper, F. J.; Battersby, A. R. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 383.

9 Scott, A. I. *Advances in Detailed Reaction Mechanisms: Mechanisms of Biological Importance.* Coxon, J. M. Ed. JAI Press: Connecticut, 1992, pp189-212.

10 (a) Battersby, A. R.; Fookes, C. J. R.; Matcham, G. W. J.; McDonald, E.; Hollenstein, R. *J. Chem. Soc., Perkin Trans. I.* **1983**, 3031; (b) Battersby, A. R.; Fookes, C. J. R.; Matcham, G. W. T.; McDonald, E. *J. Chem. Soc., Chem. Commun.* **1979**, 539; (c) Jordan, P. M.; Seehra, J. S. *FEBS Lett.* **1979**, *104*, 364; (d) Seehra, J. S.; Jordan, P. M. *J. Am. Chem. Soc.* **1980**, *102*, 6841.

11 (a) Battersby, A. R.; Fookes, C. J. R.; Matcham, G. W. T.; McDonald, E. *Nature* **1980**, *285*, 17; (b) Battersby, A. R.; Hodgson, G. L.; Hunt, E.; McDonald, E.; Saunders, J. *J. Chem. Soc., Perkin Trans. I.* **1976**, 273; (c) Battersby, A. R.; Fookes, C. J. R.; Meegan, M. J.; McDonald, E.; Wurziger, J.; Saunders, J. *J. Chem. Soc., Perkin Trans. I.* **1981**, 2786; (d) Battersby, A. R.; Fookes, C. J. R.; Gustafson-Potter, K. E.; McDonald, E.; Matcham, G. W. T. *J. Chem. Soc., Perkin Trans. I.* **1982**, 2413.

12 Merrill, B. A.; LeGoff, E. *J. Org. Chem.* **1990**, *55*, 2904.

13 Cadamuro, S.; Degani, I.; Fochi, R. Gatti, A.; Piscopo, L. *J. Chem. Soc., Perkin Trans. I.* **1993**, 2939.

14 Gharpure, M.; Stoller, A.; Bellamy, F.; Firnau, G.; Snieckus, V. *Synthesis* **1991**, 1079.

15 Casiraghi, G.; Rassu, G.; Spanu, P.; Pinna, L. *J. Org. Chem.* **1992**, *57*, 3760.

16 Loader, C. E.; Anderson, H. J. *Synthesis* **1978**, 295.

17 Drew, M. G. B.; George, A. V.; Isaacs, N. S.; Rzepa, H. S. *J. Chem. Soc., Perkin Trans. I.* **1985**, 1277.

18 (a) Altenbach, H. –J.; Constant, D.; Martin, H. –D.; Mayer, B.; Müller, M.; Vogel, E. *Chem. Ber.* **1991**, *124*, 791; (b) Tanny, S. R.; Grossman, J.; Fowler, F. W. *J. Am. Chem. Soc.* **1972**, *94*, 6495.

19 Huang, D. F.; Shen, T. Y. *Tetrahedron Lett.* **1993**, *34*, 4477.

20 Singh, S.; Basmadjian, G. P. *Tetrahedron Lett.* **1997**, *38*, 6829.

21 (a) Xu, R. X.; Anderson, H. J.; Gogan, N. J.; Loader, C. E.; McDonald, R. *Tetrahedron Lett.* **1981**, *22*, 4899; (b) Rokach, J.; Hamel, P.; Kakushima, M.; Smith, G. M. *Tetrahedron Lett.* **1981**, *22*, 4901; (c) Kashushima, M.; Hamel, P.; Frenette, R.; Rokach, J. *J. Org. Chem.* **1983**, *48*, 3214; (d) Anderson, H. J.; Loader, C. E.; Xu, R. X.; Le, N.; Gogan, N. J.; McDonald, R.; Edwards, L. G. *Can. J. Chem.* **1985**, *63*, 896; (e) Xiao, D.; Schreier, J. A.; Cook, J. H.; Seybold, P. G.; Ketcha, D. M. *Tetrahedron Lett.* **1996**, *37*, 1523.

22 For examples see: (a) Fodor, G.; Dharanipragada, R. *Nat. Prod. Reports* **1986**, *3*, 181; (b) Fodor, G.; Dharanipragada, R. *Nat. Prod. Reports* **1985**, *2*, 221; (c) Fodor, G.; Dharanipragada, R. *Nat. Prod. Reports* **1984**, *1*, 231.

23 Davies, H. M. L.; Young, W. B.; Smith, H. D. *Tetrahedron Lett.* **1989**, *30*, 4653.

24 Davies, H. M. L.; Saikali, E.; Young, W. B. *J. Org. Chem.* **1991**, *56*, 5696.

25 Mann, J.; Barbosa, L. –C. A. *J. Chem. Soc., Perkin Trans. I.* **1992**, 787.

26 Rawal, V. H.; Jones, R. J.; Cava, M. P. *J. Org. Chem.* **1987**, *52*, 19.

27 Donnhoe, T. J.; Guyo, P. M. *J. Org. Chem.* **1996**, *61*, 7664.

28 (a) Hasan, I.; Marinelli, E. R.; Lin, L. –C. C.; Fowler, F. W.; Levy, A. B. *J. Org. Chem.* **1981**, *46*, 157; (b) Chen, W.; Cava, M. P. *Tetrahedron Lett.* **1987**, *28*, 6025.

29 Tietze, L. F.; Kettschau, G.; Heitmann, K. *Synthesis* **1996**, 851.

30 Chadwick, D. J.; Willbe, C. *J. Chem. Soc., Perkin Trans. I.* **1977**, 887.

31 Gilow, H. M.; Burton, D. E. *J. Org. Chem.* **1981**, *46*, 2221.

32 Muchowski, J. M.; Solas, D. R. *J. Org. Chem.* **1984**, *49*, 203.

33 Chadwick, D. J.; Hodgson, S. T. *J. Chem. Soc., Perkin Trans. I.* **1982**, 1833.

34 Martinez, G. R.; Grieco, R. A.; Srinivasan, C. V. *J. Org. Chem.* **1981**, *46*, 3761.

35 (a) Lynch, D. R.; Snyder, S. H. *Ann. Rev. Biochem.* **1986**, *55*, 773; (b) McKelvy, J. F. *Ann. Rev. Neurosci.* **1986**, *9*, 415; (c) Neurath, H. *Science* **1984**, *224*, 350.

36 Abell, A. D. *Advances in Detailed Reaction Mechanisms: Mechanisms of Biological Importance*. Coxon, J. M. Ed. JAI Press: Connecticut, 1992, pp243-277, and references therein.

37 Rich, D. H. *Comprehensive Medicinal Chemistry: The Rational Design, Mechanistic Study and Therapeutic Application of Chemical Compounds*. Pergamon Press: Great Britain, 1990, Vol 2, pp391-441.

[38] (a) Hörl, W. H.; Heidland, A. Eds. *Proteases II: Potential Role in Health and Disease.* In *Advances in Experimental Medicine and Biology.* Plenum Press: New York, 1988, Vol 240; (b) Groutas, W. C. *Med. Res. Rev.* **1987**, *7*, 227.

[39] Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.

[40] Laskowski, M.; Tashiro, M.; Empie, M. W.; Park, S. J.; Kato, I.; Ardelt, W.; Wieczork, M. *Proteinase Inhibitors: Medical and Biological Aspects.* Springer-Verlag: Tokyo, 1983, pp55-68.

[41] (a) Schowen, R. W. *Mechanistic Principles of Enzyme Activity.* Liebman, J. F.; Greenburg, A. Eds. VCH Publishers: Weinheim, 1988, pp119-168; (b) Tsukada, H.; Blow, D. M. *J. Mol. Biol.* **1985**, *184*, 703.

[42] Bachovchin, W. W. *Biochemistry*, **1986**, *25*, 7751.

[43] Fersht, A. *Enzyme Structure and Mechanism.* W. H. Freeman and Company: New York, 1985, 2nd Edn, pp405-413.

[44] Hayakawa, Y.; Kawakami, K.; Seto, H. *Tetrahedron Lett.* **1992**, *33*, 2701.

[45] Fürstner, A.; Weintritt, H. *J. Am. Chem. Soc.* **1998**, *120*, 2817.

[46] Ortega, M. J.; Zubia, E.; Carballo, J. L.; Salvá, J. *Tetrahedron* **1997**, *53*, 331.

[47] (a) Carté, B.; Faulkner, D. J.; *J. Org. Chem.* **1983**, *48*, 2314; (b) Lidquist, N.; Fenical, W. *Experientia* **1991**, *47*, 504; (c) Kazlauskas, R.; Marwood, J. F.; Murphy, P. T.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 215; (d) Blackman, A. J.; Li, C. *Aust. J. Chem.* **1994**, *47*, 1625.

[48] (a) Wrede, F.; Hettche, O. *Ber. Dtsch. Chem. Ges. B.* **1929**, *62*, 2678; (b) Gerber, N.; Gauthier, M. J. *Appl. Environ. Microbiol.* **1979**, *37*, 1176.

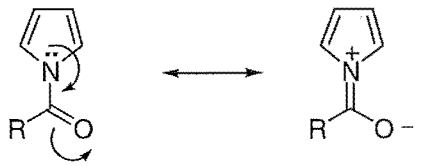
[49] (a) Matsunaga, S.; Fusetani, N.; Hashimoto, K. *Experientia* **1986**, *42*, 84; (b) Wasserman, H. H.; Friedland, D. J.; Morrison, D. A. *Tetrahedron Lett.* **1968**, *24*, 641.

# CHAPTER TWO

# SYNTHESIS AND PROPERTIES OF RING-DEACTIVED HYDROXYMETHYLPYRROLES

## 2.1 Introduction

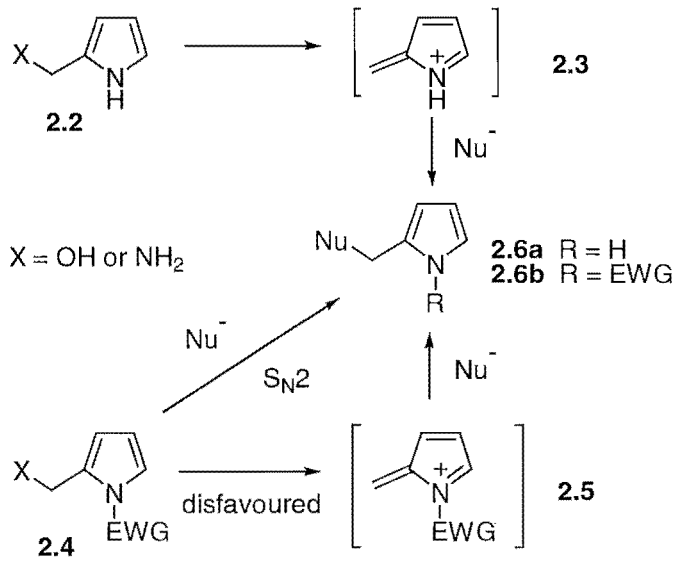
The substitution of pyrrole on nitrogen with an electron withdrawing group (EWG), such as an acyl group as in **2.1**, results in a decrease in the aromaticity of the pyrrole ring. This is due to delocalisation of the nitrogen lone pair onto the amide oxygen (see Figure 2.1).[1]




**2.1**

**Figure 2.1.** Electron delocalisation in *N*-acylpyrroles.

Similarly, it would be expected that the introduction of an electron withdrawing group onto the nitrogen of 2-substituted pyrroles of the type **2.4** would also serve to deactivate the ring system. In the absence of such deactivation pyrroles of type **2.2**, which have a potential leaving group (X) at the 2-position, readily undergo substitution at this position via the postulated azafulvene intermediate **2.3**, to give products of the type **2.6a** (Scheme 2.1).[2] However, with a deactivating EWG on the pyrrole nitrogen as in **2.4**, the analogous reaction is disfavoured due to suppression of azafulvenium **2.5** formation. In this instance, we postulate that pyrroles of type **2.4** will react with nucleophiles via $S_N2$ chemistry to give products of the type **2.6b**.




**Scheme 2.1.** Azafulvene formation of 2-substituted pyrroles.

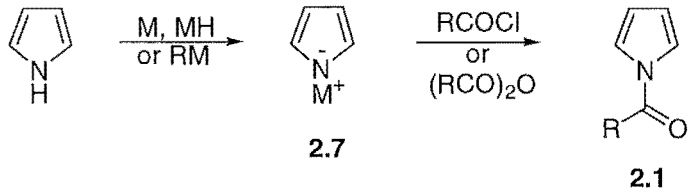
A number of electron withdrawing substituents have been utilised in order to protect and deactivate pyrrole. Examples of these groups include alkyl- and arylsulfonyls such as methanesulfonyl (mesyl), trifluoromethanesulfonyl (triflyl), phenylsulfonyl, and (4-methylphenyl)sulfonyl (tosyl), as well as alkoxycarbonyls such as *tert*-butoxycarbonyl (BOC), ethoxycarbonyl and methoxycarbonyl (see Chapter 1.3 for some examples that use ring-deactivated pyrroles as synthetic intermediates).

Accordingly, a number of methodologies have been developed to efficiently substitute the pyrrole nitrogen with an appropriate deactivating group. The following section summarises some of the approaches that have been utilised in order to effect substitution on a pyrrole nitrogen.

### Pyrrolyl anion mediated *N*-acylation of pyrrole

The p$K_a$ value for the NH proton of pyrrole has been determined spectrophotometrically by Yagil to be 17.5.[3] However, more recent measurements by Bordwell *et al.*, conducted in dimethyl sulfoxide, have set the value at 23.05.[4] Consequently, pyrrole undergoes analogous reactions to alcohols (p$K_a$ ~ 18) and alkynes

($pK_a$ ~ 22) with strong bases, to generate *N*-pyrrolyl metal salts of the type **2.7**, which can then be acylated to give products of the type **2.1** (Scheme 2.2). The more commonly used bases to effect this *N*-acylation include alkali metals such as potassium, sodium or lithium, although the use of *N*-pyrrolyl salts of silver and thallium, as well as of quaternary ammonium ions, have also been described.[5]

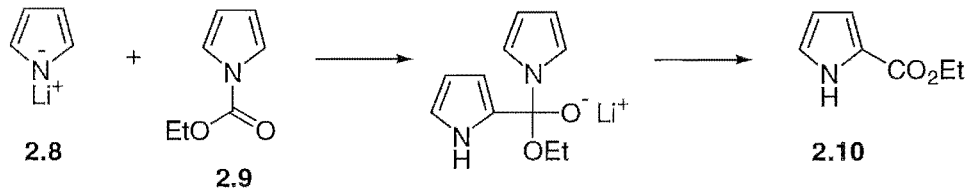



**Scheme 2.2.** Pyrrolyl anion mediated *N*-acylation of pyrrole.

The bond character of the N–M bond depends both upon the cation and upon the solvent system. With small cations such as lithium, the high charge per volume ratio, together with the close proximity of the ions, gives a strong ion pair which has a reasonably high degree of covalent character. The pyrrolyl lithium salt has therefore been described as existing as a 'solvent-separated ion-pair' in analogy to the indolyl alkali salts whose structures have been deduced from NMR spectroscopy.[6] In contrast, with the larger cations such as sodium and potassium the bonding is predominantly ionic, and in a solvent which can solvate either or both of the ions, only limited association exists between the ions. Therefore, the pyrrolyl salts of sodium and potassium are described as existing as 'contact ion-pairs'.[6]

Acylation of the pyrrolyl anion, generated from sodium or potassium, with either an acid chloride, anhydride or ester gives predominantly the *N*-substituted derivative (*eg* **2.1** in Scheme 2.2).[5,7] With the lithium derived salt, however, a mixture of *N*- and *C*-substituted derivatives is often observed. For example, reaction of the *N*-pyrrolyl lithium salt **2.8** with ethyl chloroformate leads to the formation of both the *N*- and *C*-substituted

isomers **2.9** and **2.10**, respectively. The *C*-isomer **2.10** is postulated to arise from the initially formed *N*-isomer **2.9** by the sequence shown in Scheme 2.3.[8]




**Scheme 2.3.** Formation of the *C*-substituted pyrrole **2.10**.

In contrast to the sodium or potassium salts of pyrrole, acylation of the magnesium salt of pyrrole gives predominantly the *C*-substituted derivative over the *N*-substituted derivative. However, the extent to which the *C*-acyl product prevails over the *N*-acyl product depends greatly upon both the carbonyl compound used to effect the acylation, and also on the coordinating ability of the solvent (see Chapter 3.3 for further discussion).[7,9]

*N*-Acylation of pyrrole, and of 2-formylpyrrole, has also been reported to occur in high yield through the use of the corresponding pyrrolyl thallium salts.[10] Similarly, high yields of *N*-acylpyrroles have been achieved through the use of tetrabutylammonium hydrogensulfate under phase-transfer conditions.[11]

The presence of an electron withdrawing group on the pyrrole ring serves to stabilise the generated pyrrolyl anion by increasing the electron delocalisation. This aids in the dissociation of the nitrogen-metal bond, and as a consequence, these ring-deactivated pyrroles can be more readily substituted at nitrogen, and under much milder conditions, than the corresponding non-deactivated pyrroles.[5,8,9]

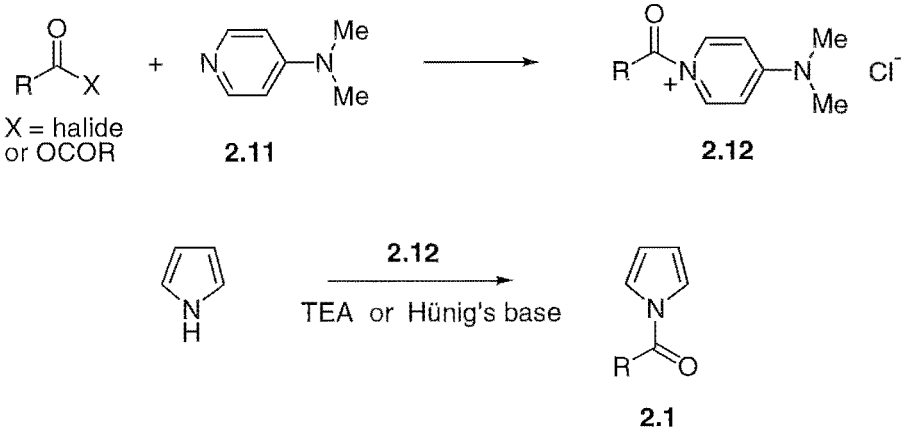
The N-substitution methodology which was used to prepare a large number of the *N*-substituted pyrrole derivatives described in this thesis was based on the work of Moon *et al.*[12] This work reported that treatment of substituted pyrrole derivatives with sodium

hydride, followed by reaction of the generated *N*-pyrrolyl anion with various acid chlorides, gave *N*-acylated pyrroles in good yields (*ca.* 75 – 80%).

**DMAP mediated *N*-acylation of pyrrole**

Steglich and Höfle[13] have identified 4-dimethylaminopyridine **2.11** (DMAP, Scheme 2.4) as a high yielding acylating catalyst (0.1 equivalents with respect to the species being acylated) which is approximately $10^4$ times more active in promoting acylation than pyridine. DMAP can either be used alone, or more commonly, with an excess of either triethylamine (TEA) or *N,N*-diisopropylethylamine (Hünig's base).

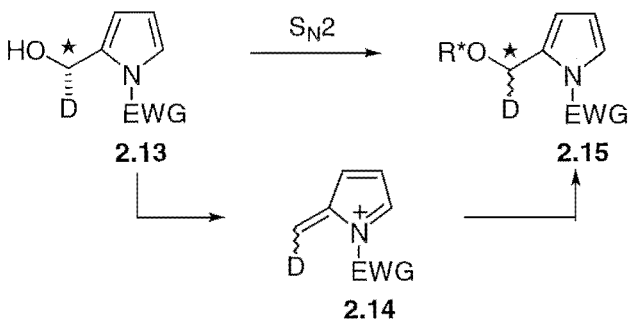
The high catalytic ability of DMAP to effect acylation is due, in part, to the formation of *N*-acylpyridinium salts **2.12** in solution, which are present as loosely-bound, and highly reactive, ion pairs.[13b]




**Scheme 2.4.** DMAP mediated *N*-acylation of pyrrole.

## 2.2. Quantifying the deactivation of pyrrole with different EWG's

An initial goal of our studies was to develop a methodology that would allow the deactivating ability of an electron withdrawing group on the pyrrole ring to be quantified. We reasoned that such an analysis would be possible by determining the extent to which azafulvene formation was suppressed by a particular deactivating group during an $S_N2$ reaction on pyrrole (see Scheme 2.5). However, in order to allow this analysis several issues pertaining to this determination first needed to be established. Of foremost importance was to develop a procedure that would readily enable the extent of azafulvene suppression during a particular reaction to be ascertained. Consequently, we decided on the use of a series of *N*-deactivated hydroxymethylpyrroles of the type **2.13**, which were stereoselectively labelled with a deuterium atom at the *exo*-methylene position (labelled ★ in Scheme 2.5), as the means by which to measure the extent of azafulvene suppression in the subsequent reaction with a nucleophile. This could be easily achieved by determining the configuration at the deuterated *exo*-methylene position, both before (*ie* **2.13**) and after substitution at ★ (*ie* **2.15**), and then comparing these results with each other, and with the corresponding unlabelled analogue, prepared as a reference sample. In this way, the configurational purity at the deuterium-labelled methylene position of **2.15** would then give an indication of the degree of azafulvene




**Scheme 2.5.** Proposed method to determine the deactivating ability of an *N*-substituent.

formation **2.14** which had occurred during this reaction. The smaller the degree of scrambling of the deuterium-label at ★ in **2.15**, the more effective the *N*-substituent would be at suppressing azafulvene formation, *ie* the greater the deactivating ability of the electron withdrawing group on the pyrrole ring.

Having established a method by which the deactivating ability of an *N*-substituent could be determined, several other key issues needed to be addressed before this analysis could be carried out. The issues which required addressing included which electron withdrawing groups were to be investigated, how the starting deuterated hydroxymethylpyrroles could be prepared in a stereoselective manner, and how these labelled hydroxymethylpyrroles, along with their corresponding reaction products, could be analysed. Finally, for this analysis to be successful we needed to select a reaction which was known to favour an $S_N2$ mechanism. This was important because such a mechanism would be likely to allow azafulvene formation **2.14** during the reaction sequence. However, the more deactivating the *N*-substituent, the greater the extent of azafulvene suppression, which would result in a smaller degree of scrambling of the deuterium-label at ★. Subsequent analysis of the configuration of these products would then allow the deactivating abilities of a range of *N*-substituents to be determined and compared. The following section summarises the ways the key issues identified above were resolved, so as to allow this analysis of the pyrrole ring-deactivation.

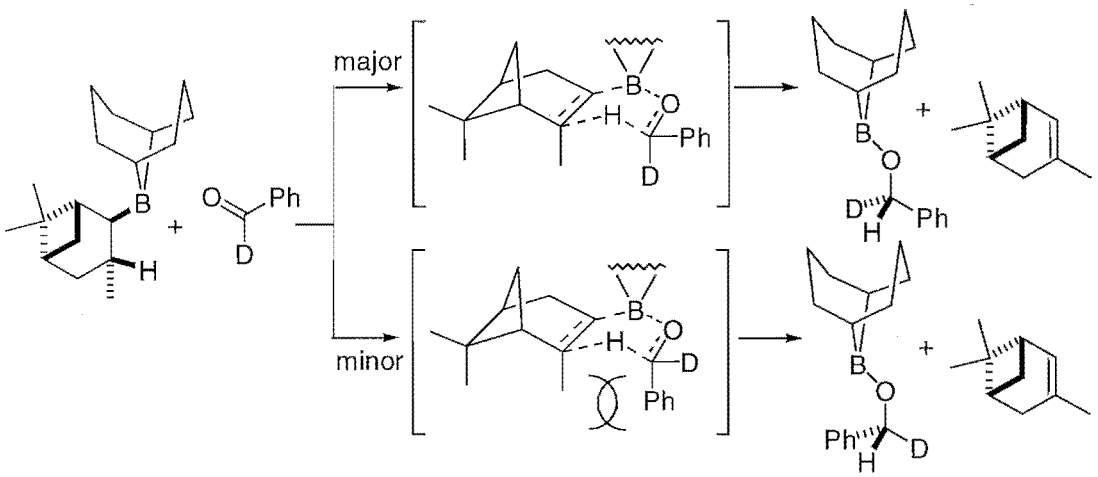
**Deactivating groups to be investigated**

The electron withdrawing substituents that were chosen to be investigated in this analysis were the trifluoromethanesulfonyl (triflyl), methanesulfonyl (mesyl), *tert*-butoxycarbonyl (BOC) and acetyl groups. These groups were selected for analysis as they were deemed as representative examples of the different classes of substituents, namely sulfonyl, alkoxycarbonyl and acyl, that had previously been employed on the pyrrole nitrogen in order to deactivate the ring system. In addition, these groups were

also of relevance to our work on developing mechanism-based inhibitors of serine proteases (see Chapter 5).

**Preparation of stereoselectively deuterium-labelled hydroxymethylpyrroles**

In order to prepare the key deuterium-labelled *N*-substituted hydroxymethylpyrroles which were to be used in the subsequent analysis, a method was required by which the *R*- and *S*-enantiomers could be synthesised stereoselectively. This could be achieved through the reduction of the corresponding deuterated *N*-substituted formylpyrrole with a chiral reducing agent such as *B*-isopinocampheyl-9-borabicyclo[3.3.1]nonane (Ipc.BBN or Alpine borane®, see Scheme 2.6).[14] Use of the reagent derived from either (–) or (+)-pinene allows access to either the *R* or the *S* product stereoselectively.




**Scheme 2.6.** Stereoselective reduction of α-deuterobenzaldehyde with Alpine borane®.

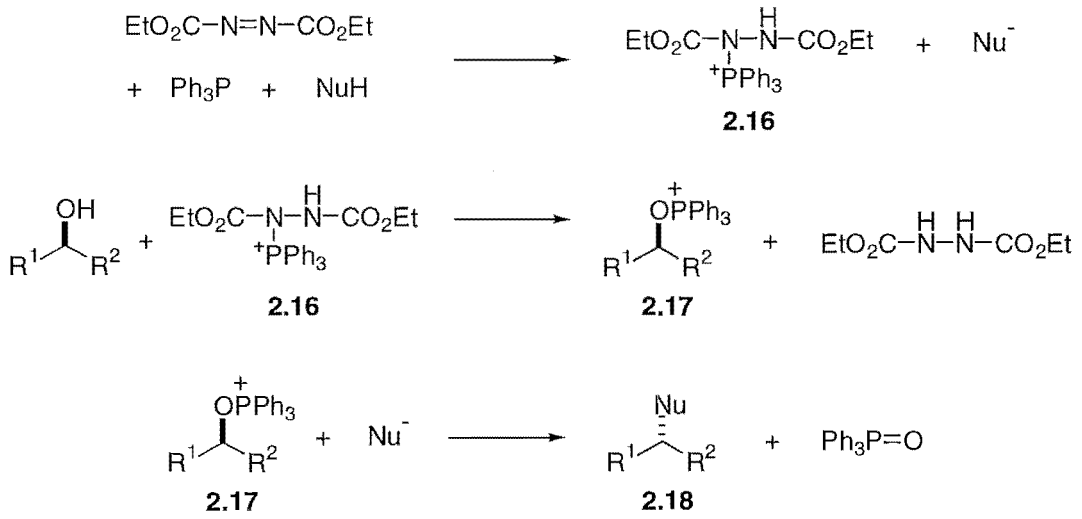
**Method for the resolution of stereochemistry at the deuterated methylene centre**

The ability to resolve the configuration at the deuterated methylene position of the labelled hydroxymethylpyrroles (*eg* **2.13**), along with their corresponding reaction products (*eg* **2.15**), was a key requirement for the subsequent analysis of pyrrole deactivation. As such, we required the introduction of a chiral functional group, or

derivatising agent, onto the pyrrole hydroxymethyl group (labelled R* in Scheme 2.5) which would allow the discrimination of the *pro*-chiral hydrogens at the *exo*-methylene position by $^1$H NMR spectral analysis. The agent which was chosen to provide this chiral functionality, and so allow discrimination at the *exo*-methylene position, was the (1*S*,4*R*)-(–)-camphanate group. This group was selected as it had been previously described by Gerlach,[15] and later by other research groups,[16] as a useful chiral derivatising agent for determining the enantiomeric purity of α-deuterated primary alcohols. Thus, substitution of the hydroxymethylpyrrole **2.13** with the (1*S*,4*R*)-(–)-camphanate group would give a derivative (*ie* **2.15**) in which the *exo*-methylene protons were distinguishable by $^1$H NMR spectroscopy.

**Selection of a reaction known to proceed via an $S_N2$ mechanism**

The reaction chosen to afford the $S_N2$ conditions necessary in this analysis was the Mitsunobu reaction, which was first reported by Mitsunobu and Yamada in 1967.[17] In this paper, the authors reported that carboxylic acids could be esterified with primary and secondary alcohols using the redox system diethyl azodicarboxylate (DEAD) and triphenylphosphine ($Ph_3P$). It was subsequently demonstrated by Mitsunobu and Eguchi[18] that this reaction generally proceeds with inversion of configuration when starting with optically pure secondary alcohols. Since this time the synthetic applications of the Mitsunobu reaction have been greatly extended to include reactions with a variety of acids (NuH in Scheme 2.7), such as imides, iminodicarbonates, sulfonamides, heterocycles, carbon acids and phenols, as well as with a range of inorganic nucleophiles such as azide, cyanide and halides.[19] The Mitsunobu reaction is believed to proceed via a three step mechanism, as detailed in Scheme 2.7 for the reaction between a nucleophile (NuH) and a chiral secondary alcohol. Initially, triphenylphosphine adds to diethyl azodicarboxylate to form a zwitterionic P-N adduct, which in the presence of the nucleophile is protonated to give the quaternary phosphonium salt **2.16**. The phosphorus group is then transferred to the alcohol to form an alkoxyphosphonium salt **2.17**, and in

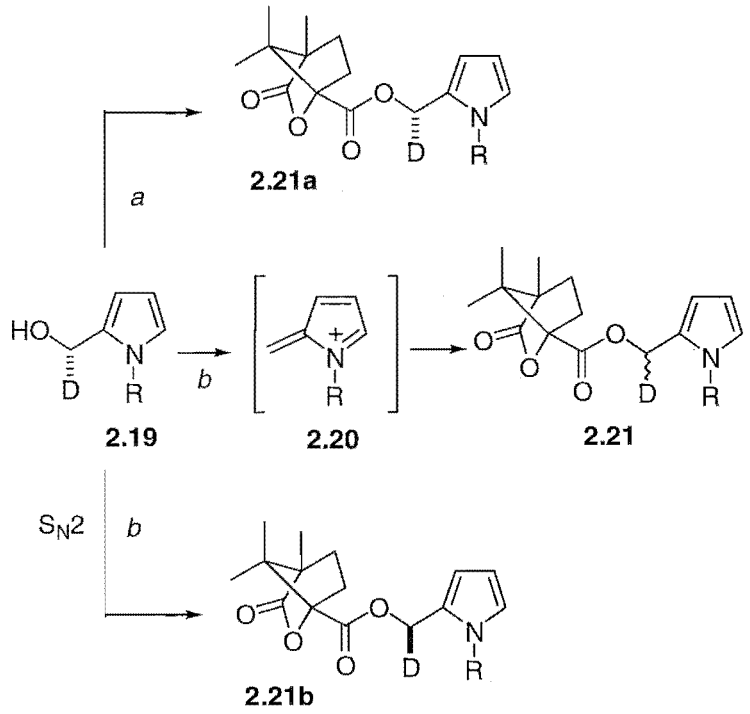



**Scheme 2.7.** Proposed mechanism of the Mitsunobu reaction.

the final step the oxyphosphonium salt is displaced by the deprotonated nucleophile ($Nu^-$) in an $S_N2$ reaction to give the inverted product **2.18**.[17-19] The Mitsunobu reaction is therefore a redox process as in this mechanism the triphenylphosphine is oxidised to triphenylphosphine oxide, while diethyl azodicarboxylate is reduced to diethyl hydrazinedicarboxylate.

Having established all the necessary requirements that would allow the analysis of the deactivating ability of an electron withdrawing group on the pyrrole ring, we therefore required a model system on which to investigate this ring-deactivation. As such, we chose to study the conversion of stereoselectively deuterium-labelled hydroxymethylpyrroles of the type **2.19** into the corresponding camphanates **2.21**, under both Mitsunobu (*ie* $S_N2$) conditions [Scheme 2.8, conditions (*b*)] and via reaction with (1*S*,4*R*)-(–)-camphanoyl chloride [Scheme 2.8, conditions (*a*)], as the means by which to analyse the deactivating abilities of various *N*-substituents (*ie* R group in **2.19**).

Accordingly, using the synthetic sequence shown in Scheme 2.8, reaction of **2.19** with (1*S*,4*R*)-(–)-camphanoyl chloride would provide reference camphanate samples for $^1H$ NMR spectral analysis, where the configurational purity at the deuterium-labelled centre of **2.21a** would be maintained and indeed measurable [Scheme 2.8, pathway (*a*)].




(*a*) DMAP, Hünig's base, (1*S*,4*R*)-(–)-camphanoyl chloride
(*b*) $Ph_3P$, DEAD, (1*S*,4*R*)-(–)-camphanic acid (Mitsunobu conditions)

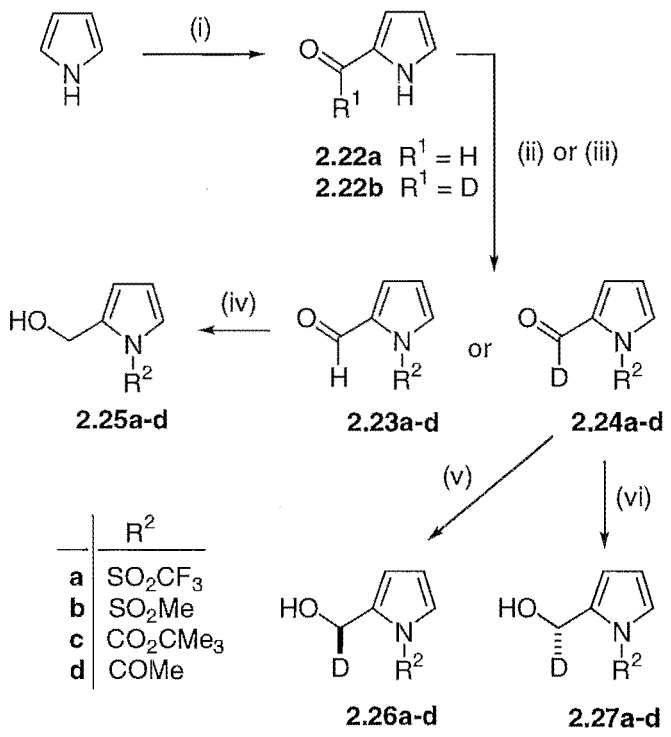
**Scheme 2.8.** Reaction sequence to assess the deactivating ability of an *N*-substituent.

However, reaction of **2.19** with (1*S*,4*R*)-(–)-camphanic acid under Mitsunobu conditions could either occur via the azafulvene **2.20**, resulting in scrambling of the deuterium-label in **2.21**, or via an $S_N2$ mechanism to give inversion of configuration at the labelled centre of the camphanate **2.21b** [Scheme 2.8, pathway (*b*)].

As has already been discussed, it was reasoned that the greater the deactivating ability of the R group on the pyrrolic nitrogen the less would be the contribution from the azafulvene mechanism. Therefore, by comparing the stereochemical integrity at the deuterium-labelled centre in **2.21**, derived from **2.19** by the Mitsunobu sequence [Scheme 2.8, reaction conditions (*b*)], with reference samples obtained from **2.19** and the corresponding unlabelled analogues by esterification with (1*S*,4*R*)-(–)-camphanoyl chloride [Scheme 2.8, reaction conditions (*a*)], the deactivating abilities of various R groups could then be assessed and quantified.

## 2.3. Preparation of starting materials and reference compounds

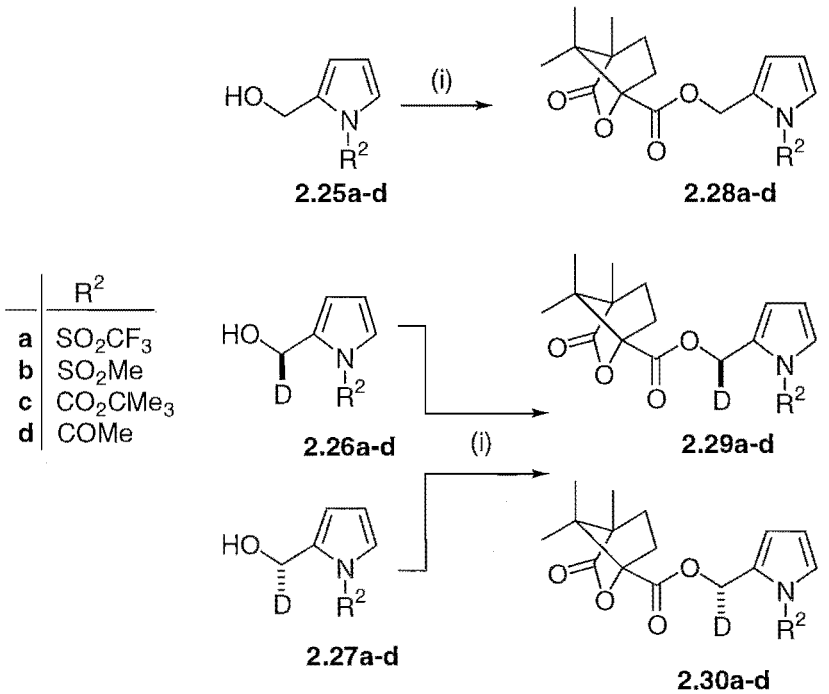
The key deuterium-labelled formylpyrrole **2.22b** was prepared in 53% yield by the Vilsmeier-Haack formylation of pyrrole,[20] using phosphorus oxychloride and *N,N*-dimethylformamide-$d_7$ (Scheme 2.9). Mass spectrometry of **2.22b** showed the compound to have an isotopic incorporation of 97.6% deuterium. The *N*-trifluoromethanesulfonyl (triflyl) protected formylpyrroles **2.23a** and **2.24a** were prepared from the corresponding formylpyrroles **2.22a** or **2.22b** in yields of 42% and 46%, respectively, by reaction with trifluoromethanesulfonic anhydride in the presence of Hünig's base, at –78 °C. The remaining *N*-protected formylpyrroles **2.23b-d** and **2.24b-d** were prepared by treating the corresponding formylpyrroles **2.22a** or **2.22b** with sodium hydride, and then reacting the generated *N*-pyrrolyl anion with either methanesulfonyl chloride, 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON®) or acetyl chloride (Scheme 2.9). In this way, the unlabelled formylpyrroles **2.23b-d** were prepared in yields of 93%, 91% and 74%, respectively, while the labelled analogues **2.24b-d** were prepared in yields of 92%, 87% and 94%, respectively. The unlabelled hydroxymethylpyrroles **2.25a-d** were then readily prepared, in yields of 90%, 91%, 91% and 98%, respectively, by reducing the *N*-protected formylpyrroles **2.23a-d** with zinc borohydride. The corresponding labelled analogues, **2.26a-d** and **2.27a-d**, were prepared by reducing the deuterated formylpyrroles **2.24a-d** with either *S*-Alpine borane®, or *R*-Alpine borane®, respectively (Scheme 2.9). However, only the hydroxymethylpyrroles **2.26b**, **2.27b** and **2.27d** were able to be isolated as clean products, in yields of 73%, quantitative and 85%, respectively. The other hydroxymethylpyrroles were unable to be fully purified from the by-products of the reduction reaction, and so were utilised in the subsequent steps without further purification. In each of these cases, however, the desired hydroxymethylpyrrole was the predominant compound (*ca.* >80% by $^1$H NMR spectroscopy).




**Scheme 2.9.** *Reagents and conditions*: (i) $POCl_3$, DMF-$d_7$, 1,2-DCE (53%); (ii) $(CF_3SO_2)_2O$, Hünig's base, $CH_2Cl_2$, –78 °C (**2.23a**, 42%), (**2.24a**, 46%); (iii) NaH, THF then either $MeSO_2Cl$ (**2.23b**, 93%), (**2.24b**, 92%) or BOC-ON® (**2.23c**, 91%), (**2.24c**, 87%) or MeCOCl (**2.23d**, 74%), (**2.24d**, 94%); (iv) $Zn(BH_4)_2$, $Et_2O$, 0 °C (**2.25a**, 90%), (**2.25b**, 91%), (**2.25c**, 91%), (**2.25d**, 98%); (v) *S*-Alpine borane®, THF, rt (**2.26a,c-d**, see text), (**2.26b**, 73%); (vi) *R*-Alpine borane®, THF, rt (**2.27a,c**, see text), (**2.27b**, quant.), (**2.27d**, 85%).

Next we turned our attention to the synthesis of the key unlabelled and labelled camphanates **2.28a-d**, **2.29a-d** and **2.30a-d**, respectively, which would serve as reference compounds in the subsequent $^1$H NMR spectral analysis. Consequently, the unlabelled hydroxymethylpyrroles **2.25a-d** were esterified with (1*S*,4*R*)-(–)-camphanoyl chloride using standard procedures to give the camphanates **2.28a-d** in yields of 95%, 86%, 76% and 99%, respectively (Scheme 2.10). The $^1$H NMR spectra of these reference compounds **2.28a-d** revealed that, in each case, the pyrrole *exo*-methylene group was observed as an AB quartet, resonating at δ 5.32 and 5.37, 5.35 and 5.42, 5.41 and 5.45,

and 5.44 and 5.49, respectively. The camphanates **2.29a-d** and **2.30a-d** were similarly prepared by esterification of the corresponding labelled hydroxymethylpyrroles **2.26a-d** and **2.27a-d** with (1*S*,4*R*)-(–)-camphanoyl chloride (Scheme 2.10). In this way, the camphanates **2.29a-d**, derived from the *S*-Alpine borane® sequence, were prepared in yields of 40%, 53%, 69% and 35%, respectively, while the camphanates **2.30a-d**, derived from the *R*-Alpine borane® sequence, were prepared in yields of 53%, 31%, 82% and 11%, respectively. The $^1$H NMR spectra of the pyrrole *exo*-methylene group of these deuterium-labelled analogues **2.29a-d** and **2.30a-d** were observed as singlets with distinguishable chemical shifts at δ 5.31 and 5.33, 5.35 and 5.41, 5.41 and 5.42, and 5.43 and 5.44, respectively. Furthermore, in the case of the *S*-Alpine borane® derived camphanates **2.29a-d**, the configurational purities at the deuterated methylene centre were observed to be ~9:1, while the camphanates **2.30a-d** derived from the *R*-Alpine

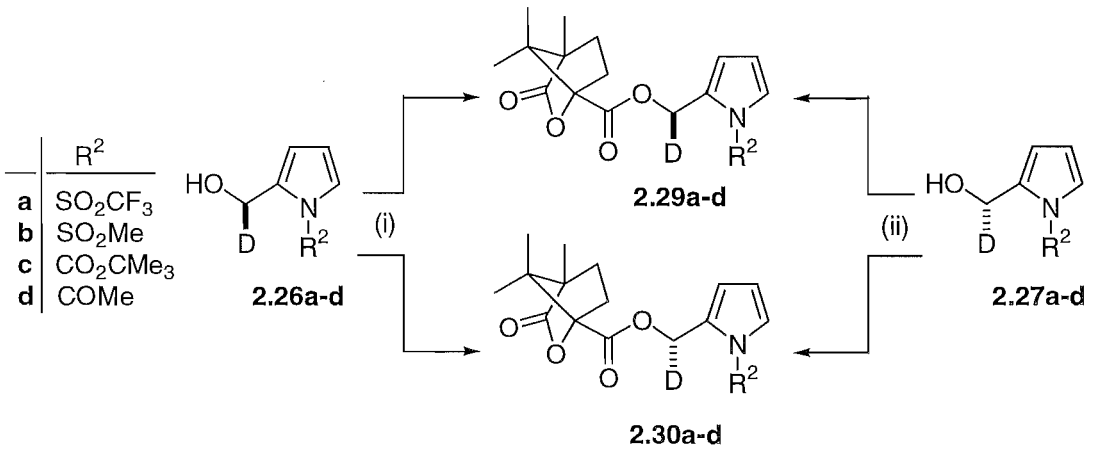



**Scheme 2.10.** *Reagents and conditions*: (i) DMAP, Hünig's base, (1*S*,4*R*)-(–)-camphanoyl chloride, $CH_2Cl_2$ (**2.28a**, 95%), (**2.28b**, 86%), (**2.28c**, 76%), (**2.28d**, 99%), (**2.29a**, 40%), (**2.29b**, 53%), (**2.29c**, 69%), (**2.29d**, 35%), (**2.30a**, 53%), (**2.30b**, 31%), (**2.30c**, 82%), (**2.30d**, 11%).

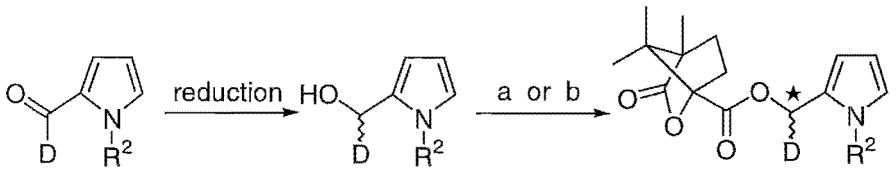
borane® sequence were observed to have slightly higher configurational purities of ~15:1 to >19:1. Therefore, as the esterification reaction has no effect on the configuration at the deuterated methylene position of the precursor hydroxymethylpyrroles, the configurational purities of **2.26a-d** and **2.27a-d** can also be inferred as being ~9:1 and ~15:1 to >19:1, respectively. See the following section for the detailed analysis of the configurational purities of these camphanate derivatives.

## *2.4. Deactivation study and configurational analysis*

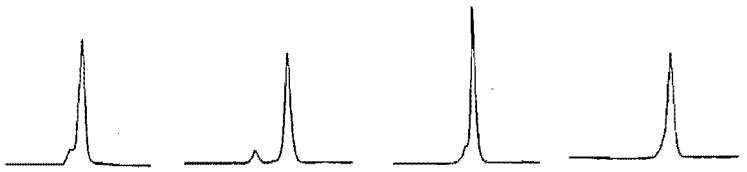
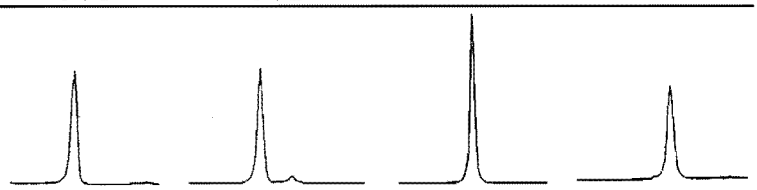
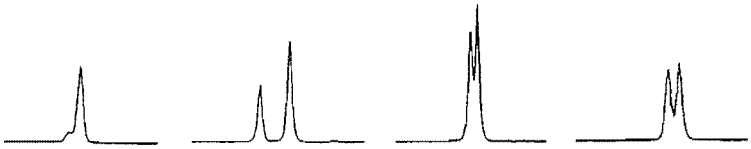
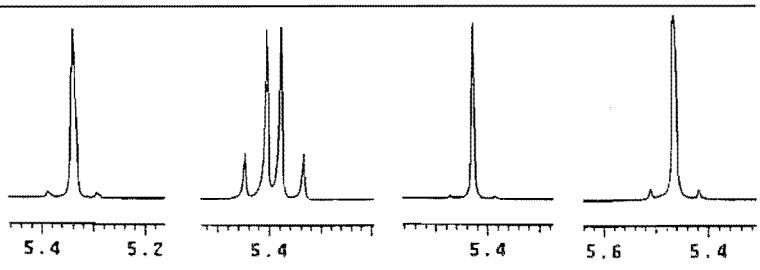
In the crucial deactivation study experiments, separate samples of the deuterated hydroxymethylpyrroles **2.26a-d**, of known configurational purity, were treated with (1*S*,4*R*)-(–)-camphanic acid under Mitsunobu conditions to give mixtures of the corresponding camphanates **2.29a-d** and **2.30a-d** in yields of 31%, 54%, 46% and 43%, respectively (Scheme 2.11). The results of the $^1$H NMR spectral analysis of the camphanates from these reactions, **2.29a-d** and **2.30a-d**, are given in Table 2.1, second




**Scheme 2.11.** *Reagents and conditions*: (i) $Ph_3P$, DEAD, (1*S*,4*R*)-(–)-camphanic acid (**2.29a/2.30a**, 31%), (**2.29b/2.30b**, 54%), (**2.29c/2.30c**, 46%), (**2.29d/2.30d**, 43%); (ii) $Ph_3P$, DEAD, (1*S*,4*R*)-(–)-camphanic acid (**2.29a/2.30a**, 32%), (**2.29b/2.30b**, 72%), (**2.29c/2.30c**, 36%), (**2.29d/2.30d**, 77%).




**2.24a-d** **2.26a-d, 2.27a-d** **2.29a-d (*R*), 2.30a-d (*S*)**

| | | $R^2$ substituent | | | |
|---|---|---|---|---|---|
| reducing agent | camphanate formation | $-SO_2CF_3$ **2.30a : 2.29a** | $-SO_2Me$ **2.30b : 2.29b** | -BOC **2.30c : 2.29c** | -COMe **2.30d : 2.29d** |
| S-Alpine borane® | a |  ~1 : 9 |  ~1 : 9 |  ~1 : 9 |  ~1 : 9 |
| | b |  ~5 : 1 |  ~3 : 2 |  ~5 : 4 |  ~1 : 1 |
| R-Alpine borane® | a |  >19 : 1 |  ~15 : 1 |  >19 : 1 |  >19 : 1 |
| | b |  ~1 : 6 |  ~2 : 3 |  ~4 : 5 |  ~1 : 1 |
| | | **2.28a** | **2.28b** | **2.28c** | **2.28d** |
| $Zn(BH_4)_2$[c] | a |  5.4 5.2 |  5.4 |  5.4 |  5.6 5.4 |

(a) DMAP, Hünig's base, (1*S*,4*R*)-(–)-camphanoyl chloride

(b) $Ph_3P$, DEAD, (1*S*,4*R*)-(–)-camphanic acid (Mitsunobu conditions)

(c) reaction sequence was performed using unlabelled **2.25a-d** to give **2.28a-d**

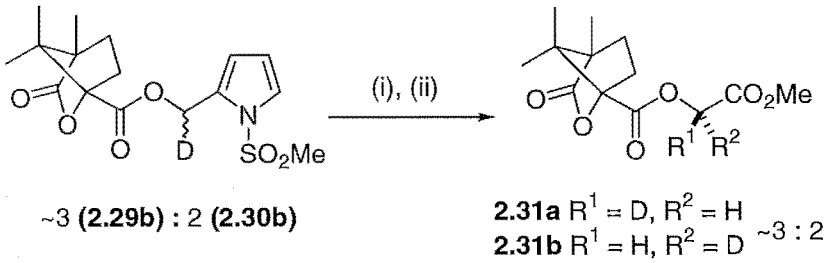
**Table 2.1.** $^1$H NMR resonances ($CDCl_3$) for the *exo*-methylene ( ★ ) of **2.28**, **2.29**, **2.30**.

row of spectra. The ratio of **2.30** to **2.29**, observed on reaction of the *N*-trifluoromethanesulfonyl (triflyl), *N*-methanesulfonyl (mesyl), *N-tert*-butoxycarbonyl (BOC) and *N*-acetyl series, were ~5:1, ~3:2, ~5:4 and ~1:1, respectively. In comparison, the initial ratios of **2.27a-d** and **2.26a-d** had been determined to be ~1:9 by analysis of the reference camphanate samples (see Scheme 2.10 and Table 2.1, first row of spectra). The major isomers formed in these reactions, the camphanates **2.30a-c**, are the products of an inversion of configuration due to an $S_N2$ displacement on **2.26a-c** under the Mitsunobu conditions (Scheme 2.11). The higher the proportion of configuration **2.30** relative to configuration **2.29** in these products, the greater has been the effectiveness of the electron withdrawing group in disfavouring reaction via the azafulvenium intermediate **2.20** (see Scheme 2.8). The trifluoromethanesulfonyl group was found to be most effective in suppressing this azafulvenium formation, with the *N*-triflyl deactivated hydroxymethylpyrrole **2.26a** undergoing substitution via an $S_N2$ mechanism almost exclusively to give **2.30a**. At the other extreme, the *tert*-butoxycarbonyl and acetyl groups were found to be much less effective at deactivating the pyrrole ring, with the *N*-BOC and *N*-acetyl hydroxymethylpyrroles, **2.26c** and **2.26d**, undergoing a substantial amount of substitution via an azafulvenium mechanism.

In the complementary deactivation study experiments, samples of the deuterated hydroxymethylpyrroles **2.27a-d**, of known configurational purity, were treated with (1*S*,4*R*)-(–)-camphanic acid under Mitsunobu conditions as before, to give mixtures of the corresponding camphanates **2.29a-d** and **2.30a-d** in yields of 32%, 72%, 36% and 77%, respectively (Scheme 2.11). The ratio of **2.30** to **2.29** observed on reaction of the *N*-triflyl, *N*-mesyl, *N*-BOC and *N*-acetyl series, as given in Table 2.1, fourth row of spectra, were ~1:6, ~2:3, ~4:5 and ~1:1, respectively. In comparison, the initial ratios of **2.27a-d** and **2.26a-d** had been determined to be ~15:1 to >19:1 by analysis of the reference camphanate samples (see Scheme 2.10 and Table 2.1, third row of spectra).

Finally, the absolute configurations of **2.29a-d** and **2.30a-d**, although being consistent with literature reports for Alpine borane® reductions,[14] were confirmed by

ozonising a sample containing a ~3:2 mixture of **2.29b** and **2.30b** by the methodology of Klein and Steinmetz.[21] The resulting acid was trapped with an excess of diazomethane to give a ~3:2 mixture of **2.31a** and **2.31b** in an overall yield of 39% for both steps (Scheme 2.12). A $^1$H NMR spectrum of this mixture of **2.31a** and **2.31b** was consistent with data from authentic samples of **2.31** and with the unlabelled analogue.[22]




~3 **(2.29b)** : 2 **(2.30b)**

**2.31a** $R^1$ = D, $R^2$ = H
**2.31b** $R^1$ = H, $R^2$ = D ~3 : 2

**Scheme 2.12.** *Reagents and conditions*: (i) $O_3$, –78 °C; (ii) $CH_2N_2$, MeOH (39% for 2 steps).

## 2.5. Conclusion

A new methodology was developed which enabled the deactivating ability of an electron withdrawing group on the pyrrole ring to be quantified. This was achieved by reacting a series of chiral deuterium-labelled *N*-substituted hydroxymethylpyrroles of the type **2.13**, with the chiral resolving agent (1*S*,4*R*)-(–)-camphanic acid under Mitsunobu (*ie* $S_N2$) conditions (Scheme 2.8). By the subsequent analysis of the configurational purities at the deuterated *exo*-methylene centres of these product camphanates by $^1H$ NMR spectroscopy, and comparison of these results with previously prepared reference camphanate samples of known configurational purity, the degree of azafulvenium suppression during the $S_N2$ reaction was able to be determined. This methodology therefore enables the effectiveness of a particular electron withdrawing group to deactivate a pyrrole ring to be quantified, and also gives a method by which the deactivating abilities of a range of substituents can be compared.

The conclusion from these labelling experiments is that the order for the deactivating ability of the pyrrole *N*-protecting groups considered in this study is triflyl > mesyl > BOC ≈ acetyl. Therefore, $S_N2$ displacements at the hydroxymethyl group of compounds of the type **2.4** (Scheme 2.1) are favoured by use of *N*-triflyl as the deactivating group and Mitsunobu reaction conditions. The suppression of contributions to the reaction from azafulvenium intermediates **2.5** is less effective with other deactivating groups, with the *tert*-butoxycarbonyl (BOC) and acetyl groups having little or no effect on azafulvenium suppression. An *N*-triflyl group should, therefore, be employed when maximum deactivation of a pyrrole ring is desired. It has been assumed that the introduction of an electron withdrawing group onto a pyrrole nitrogen, as in **2.4**, suppresses the formation of highly reactive azafulvenium intermediates **2.5**, but until now little direct evidence for this has been available.

## 2.6. References for chapter two


1 Jones, A. R.; Bean, G. P. Eds. *The Chemistry of Pyrroles.* Academic Press: London, 1977, pp283-289.

2 Barcock, R. A.; Moorcroft, N. A.; Storr, R. C. *Tetrahedron Lett.* **1993**, *34*, 1187.

3 Yagil, G. *Tetrahedron* **1967**, *23*, 2855.

4 Bordwell, F. G.; Drucker, G. E.; Fried, H. E. *J. Org. Chem.* **1981**, *46*, 632.

5 Jones, A. R.; Bean, G. P. Eds. *The Chemistry of Pyrroles.* Academic Press: London, 1977, pp173-187.

6 Reinecke, M. G.; Sebastian, J. F.; Johnson, H. W.; Pyun, C. *J. Org. Chem.* **1971**, *36*, 3091.

7 Jones, R. A. *Comprehensive Heterocyclic Chemistry.* Katritzky, A. R.; Rees, C. W. Eds. Pergamon Press: Oxford, 1984, Vol 4, pp235-240.

8 Wang, N. –C.; Anderson, H. J. *Can. J. Chem.* **1977**, *55*, 4103.

9 Anderson, H. J.; Loader, C. E. *Pyrroles.* Jones, R. A. Ed. John Wiley & Sons: New York, 1990, Vol 48, Part 1, pp474-483.

10 Candy, C. F.; Jones, R. A.; Wright, P. H. *J. Chem. Soc. C.* **1970**, 2563.

11 (a) Demopoulos, B. J.; Anderson, H. J.; Loader, C. E.; Faber, K. *Can. J. Chem.* **1983**, *61*, 2415; (b) Faber, K.; Anderson, H. J.; Loader, C. E.; Daley, A. S. *Can. J. Chem.* **1984**, *62*, 1046.

12 Moon, M. W.; Bell, L. T.; Webster, D. M. *J. Org. Chem.* **1974**, *39*, 315.

13 (a) Steglich, W.; Höfle, G. *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 981; (b) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem. Int. Ed. Engl.* **1978**, *17*, 569.

14 Midland, M. M.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352.

15 (a) Gerlach, H. *Helv. Chim. Acta* **1966**, *49*, 2481; (b) Gerlach, H.; Zagalak, B. *J. Chem. Soc., Chem. Commun.* **1973**, 274.

16 (a) Schwab, J. M. *J. Am. Chem. Soc.* **1981**, *103*, 1876; (b) Schwab, J. M.; Li, W.; Thomas, L. P. *J. Am. Chem. Soc.* **1983**, *105*, 4800; (c) Schwab, J. M.; Ray, T.; Ho, C. K. *J. Am. Chem. Soc.* **1989**, *111*, 1057; (d) Shapiro, S.; Arunachalam, T.; Caspi, E. *J. Am. Chem. Soc.* **1983**, *105*, 1642.

17 Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380.

18 Mitsunobu, O.; Eguchi, M. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 3427.

19 For recent reviews of the Mitsunobu reaction and its synthetic applications see: (a) Mitsunobu, O. *Synthesis* **1981**, 1; (b) Hughes, D. L. *Org. React.* **1992**, *42*, 335; (c) Hughes, D. L. *Org. Prep. Proced. Int.* **1996**, *28*, 165.

20 Silverstein, R. M.; Ryskiewicz, E. E.; Willard, C. *Org. Synth., Coll. Vol.* **1963**, *4*, 831.

21 Klein, H.; Steinmetz, A. *Tetrahedron Lett.* **1975**, 4249.

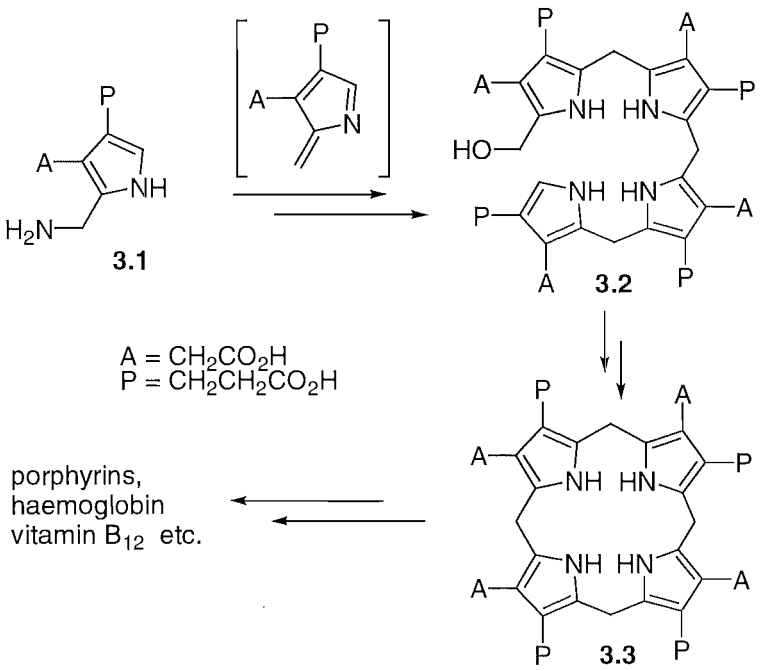
22 (a) Schauder, J-R.; Jendrezejewski, S.; Abell, A. D.; Hart, G. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1987**, 436; (b) Arigoni, D.; Besmer, P. see Besmer, P. Diss. ETH, No 4435, 1970.

# CHAPTER THREE

# SYNTHESIS OF DIPYRROMETHANES: AN APPLICATION OF DEACTIVATED HYDROXYMETHYLPYRROLES

## 3.1. Introduction

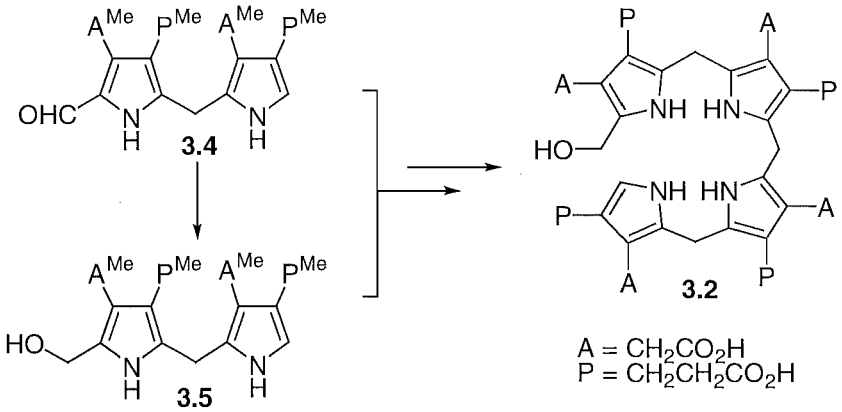
Pyrroles are the key building blocks to a large number of biomolecules which are essential for life, such as porphyrins, haemoglobin and vitamin $B_{12}$. These compounds are synthesised in nature via an initial azafulvene-based tetramerisation of the pyrrole precursor porphobilinogen (PBG) **3.1** to give hydroxymethylbilane (HMB) **3.2**, which is subsequently cyclised, also by azafulvene chemistry, to give uroporphyrinogen III (uro'gen III) **3.3** (see Chapter 1.2 for more details of the biosynthesis of uro'gen III). At this point the biosynthetic pathway diverges, and uro'gen III is transformed into the range of tetrapyrrole-based compounds found in nature (Scheme 3.1).[1]




**Scheme 3.1.** Biosynthetic pathway of tetrapyrrole-based compounds.

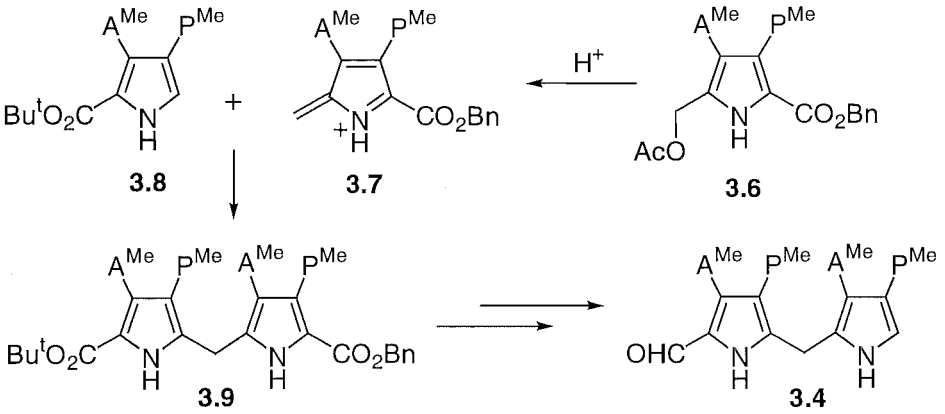
Consequently, due to their importance in a vast array of biological processes, much research has been focussed on unravelling the mechanisms by which these

important tetrapyrrole-based biomolecules are synthesised in nature.[2] In chemistry, bilanes, such as HMB **3.2**, can be prepared by coupling together two dipyrromethanes, such as **3.4** and **3.5**, under acidic conditions (Scheme 3.2).[3]



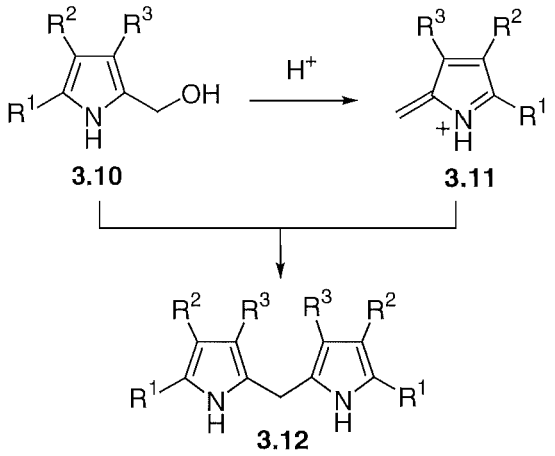

**Scheme 3.2.** Acid-catalysed synthesis of hydroxymethylbilane **3.2**.

Accordingly, dipyrromethanes are key synthetic intermediates in the preparation of bilanes. As a result, the preparation of dipyrromethanes has been the focus of much research, and various synthetic methodologies to these compounds have been reported in the literature.[3,4] What follows is a brief summary of some of the methods that have been employed. The most common approach to preparing dipyrromethanes involves the use of acid catalysed electrophilic substitution. In this method, an α-unsubstituted pyrrole, such as **3.8**, is reacted with an azafulvene of the type **3.7** to give a dipyrromethane of the type **3.9** (Scheme 3.3).[3] This azafulvene can be generated *in situ* from either an acetoxymethylpyrrole (*eg* **3.6**), a hydroxymethylpyrrole, or a chloromethylpyrrole under acidic conditions.[4]




**Scheme 3.3.** Synthesis of a dipyrromethane via azafulvene chemistry.

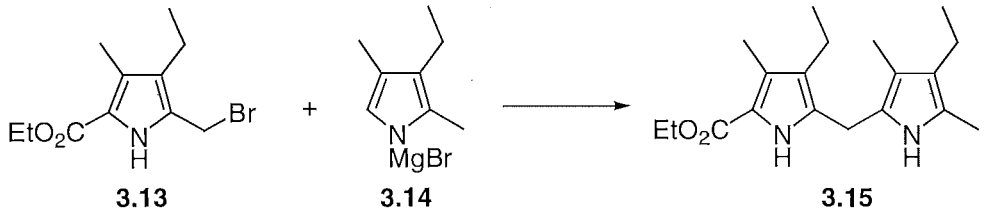
Similarly, in an analogous acid-catalysed procedure, electrophilic attack at the substituted α-position of a hydroxymethylpyrrole of the type **3.10** by its resultant azafulvene **3.11**, followed by elimination of formaldehyde, leads to the formation of symmetrical dipyrromethanes of the type **3.12** (Scheme 3.4).[4,5]




**Scheme 3.4.** Synthesis of a symmetrical dipyrromethane via azafulvene chemistry.

The preparation of dipyrromethanes under non-acidic conditions has been much less studied, with only a single report appearing in 1929.[6] Here, a tetra-substituted

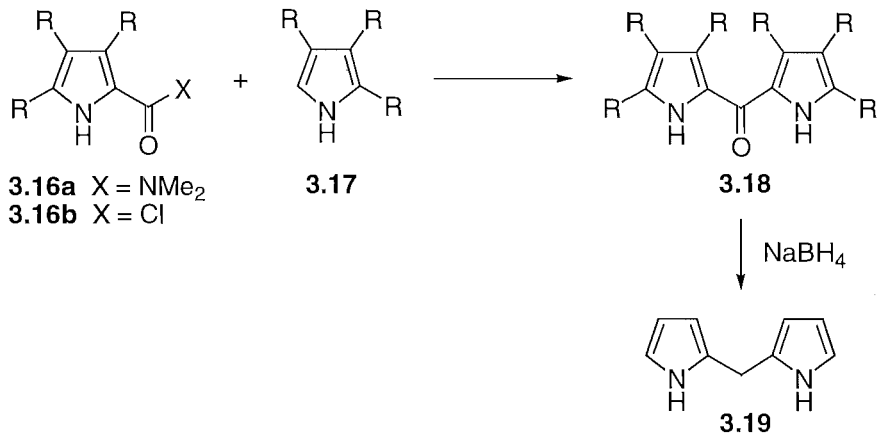
bromomethylpyrrole **3.13** was coupled with the *N*-magnesio pyrrole **3.14** to give the dipyrromethane **3.15** in 78% yield (Scheme 3.5).




**3.13** **3.14** **3.15**

**Scheme 3.5.** Pyrrolylmagnesium-mediated preparation of dipyrromethane **3.15**.

Finally, the synthesis of the simple dipyrromethane **3.19** has also been achieved by the reduction of the corresponding dipyrroketone **3.18** (R = H) with sodium borohydride (Scheme 3.6).[7] Dipyrroketones of the type **3.18** are in turn prepared by reacting an α-unsubstituted pyrrole of the type **3.17** with either a 2-(*N*,*N*-dimethylcarboxamido)pyrrole **3.16a** via a modified Vilsmeier-Haack reaction, or with a pyrrole-2-carbonyl chloride **3.16b** using standard acylation methods.[8]

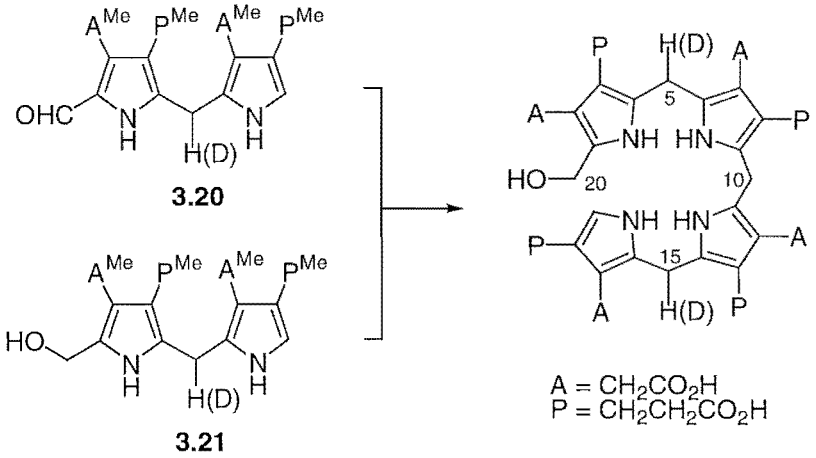



**3.16a** X = $NMe_2$
**3.16b** X = Cl

**3.17** **3.18** **3.19**

**Scheme 3.6.** Synthesis of dipyrroketones and subsequent reduction to dipyrromethanes.

In order to enable some of the key stereochemical issues associated with the biosynthesis of porphyrins and related compounds to be investigated, a chemical marker or label, with known configuration, is required in the precursor molecules. A preliminary report on this topic was published by Battersby *et al.* in 1987.[9] Consequently, by determining the position and configuration of this label in the final product, mechanistic information about the biochemical transformation can be attained. The dipyrromethanes **3.20** and **3.21**, which are stereoselectively labelled with a deuterium atom at the interpyrrolic methylene position, would therefore be invaluable derivatives in this context. Coupling these dipyrromethanes would enable the synthesis of $C_5$ and/or $C_{15}$ deuterium-labelled hydroxymethylbilanes (see Scheme 3.7). The $C_{20}$ deuterium-labelled hydroxymethylbilane has already been reported by Battersby *et al.*[9]




**Scheme 3.7.** Proposed method for the synthesis of $C_5$ and/or $C_{15}$ deuterium-labelled hydroxymethylbilanes.

However, the existing acid-catalysed azafulvene-based preparations of dipyrromethanes described above would almost certainly prove unsatisfactory in the preparation of chiral deuterium-labelled dipyrromethanes of the type **3.20** and **3.21**, since azafulvene formation would lead to the scrambling of the deuterium-label at the interpyrrolic methylene position. Likewise, the dipyrroketone reduction method would

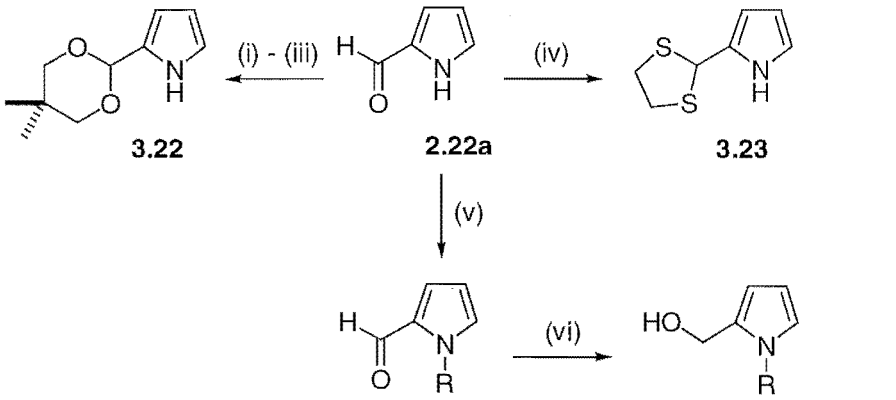
also be unsatisfactory, since the stereoselective incorporation of a deuterium atom into the interpyrrolic methylene position would not be trivial. The method of most promise in our opinion, therefore, was the nucleophilic displacement reaction using a pyrrolylmagnesium salt. This methodology has the advantage of both avoiding acidic conditions which could lead to azafulvene formation, and also could allow for the preparation of unsymmetrical dipyrromethanes.

Accordingly, the initial aim of the research described in this chapter was to further investigate this pyrrolylmagnesium-based preparation of dipyrromethanes, and to extend this methodology to the synthesis of dipyrromethanes bearing an aldehyde functionality at the α-position. For the sake of synthetic ease, initial work in this area was to be carried out on a simplified system, using pyrroles unsubstituted at the 3- and 4-positions. α-Formyldipyrromethanes were selected for synthesis because the 2-formyl group provides a suitable site for the subsequent elaboration of these compounds into bilanes of the type **3.2**, as shown in Scheme 3.2. A potential application of this methodology would be the synthesis of deuterium-labelled dipyrromethanes of the type **3.20** and **3.21**. These compounds could then be transformed into labelled hydroxymethylbilane and uro'gen III, and so be used to determine important mechanistic information about key enzymes in the biosynthesis of porphyrins and related compounds.

## *3.2. Synthesis of starting materials*

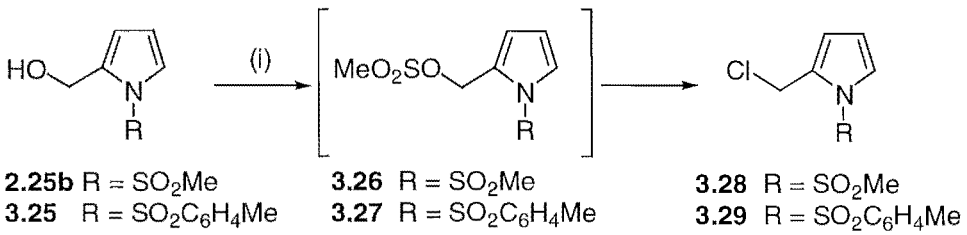
The key formyl-protected starting materials, **3.22** and **3.23**, were prepared from pyrrole-2-carboxaldehyde **2.22a** (Scheme 3.8) using standard literature procedures. The dioxolanylpyrrole **3.22** was prepared in three steps in 58% overall yield by the indirect method described by Loader and Anderson,[10] while the dithiolanylpyrrole **3.23** was prepared in 92% yield by the method described by Wallace *et al.*[11] These compounds were used to prepare the *N*-magnesio pyrroles required for the couplings. The *N*-

methanesulfonyl-2-hydroxymethylpyrrole **2.25b** was prepared from **2.22a** by N-mesylation followed by reduction, as described in Chapter 2. Compound **3.25** was similarly prepared from pyrrole-2-carboxaldehyde **2.22a** in 90% yield (2 steps) by N-substitution with tosyl chloride to give **3.24**, followed by zinc borohydride reduction of the formyl group (Scheme 3.8).




**Scheme 3.8.** *Reagents and conditions*: (i) NaH, THF then $ClCO_2Et$; (ii) $Me_2C(CH_2OH)_2$, *p*-TsOH, 1,2-DCE, reflux; (iii) NaOH, MeOH-$H_2O$, reflux (58% for 3 steps); (iv) $HSCH_2CH_2SH$, $PhNH_2$, MeOH (92%); (v) NaH, THF then either $MeSO_2Cl$ (**2.23b**, 93%) or *p*-$MeC_6H_4SO_2Cl$ (**3.24**, 99%); (vi) $Zn(BH_4)_2$ (**2.25b**, 91%), (**3.25**, 91%).

The *N*-substituted-2-chloromethylpyrroles **3.28** and **3.29** were prepared in yields of 87% and 83% from their respective hydroxymethylpyrroles **2.25b** and **3.25** by treatment with methanesulfonyl chloride and Hünig's base. These chloromethylpyrroles




**Scheme 3.9.** *Reagents and conditions*: (i) Hünig's base, $MeSO_2Cl$ then 10% aq. HCl (**3.28**, 87%), (**3.29**, 83%).

provided the alkylating agents for the reaction with the *N*-magnesio pyrroles described above. The *O*-mesylates **3.26** and **3.27** were postulated as the intermediates in this chlorination reaction, but were unable to be isolated due to their rapid *in situ* reaction with chloride ion.

In order to establish whether compounds **3.26** and **3.27** were intermediates to the chloromethylpyrroles **3.28** and **3.29**, respectively, the reaction of **2.25b** with methanesulfonyl chloride was followed by low temperature $^1$H NMR spectroscopy. Spectral analysis showed that the *O*-mesylate **3.26** was indeed formed upon the addition of methanesulfonyl chloride to a mixture of the hydroxymethylpyrrole **2.25b** and Hünig's base in $CDCl_3$ at –20 °C. However, the chloromethylpyrrole **3.28** was observed to form concurrently. The course of the reaction was able to be followed by observing the *exo*-methylene resonances at δ 4.74, 4.91 and 5.38 for the hydroxymethyl, chloromethyl and *O*-mesylpyrroles, respectively. The relative amounts of the pyrroles **2.25b**, **3.26** and **3.28** observed during the course of the reaction, as determined by integration of the repective *exo*-methylene resonances, are detailed in Table 3.1.

| | Relative % of each compound | | |
|---|---|---|---|
| Time (min) | **2.25b** | **3.26** | **3.28** |
| Initial | 100 | 0 | 0 |
| 0 | 40 | 50 | 10 |
| 2.5 | 10 | 60 | 30 |
| 5 | 0 | 50 | 50 |
| 10 | 0 | 30 | 70 |
| 15 | 0 | 20 | 80 |
| 20 | 0 | 10 | 90 |
| 30 | 0 | 5 | 95 |
| 45 | 0 | 2.5 | 97.5 |
| 60 | 0 | 0 | 100 |

**Table 3.1.** Relative amounts of the pyrroles **2.25b**, **3.26** and **3.28** observed by $^1$H NMR spectroscopy during the course of the reaction.

From the results detailed in Table 3.1 it can be seen that the hydroxymethylpyrrole **2.25b** was rapidly converted into the *O*-mesylate **3.26** within 5 min at –20 °C. However, the subsequent conversion of the *O*-mesylate **3.26** into the chloromethylpyrrole **3.28** occurred at a slower rate, and was not complete until around 60 min.

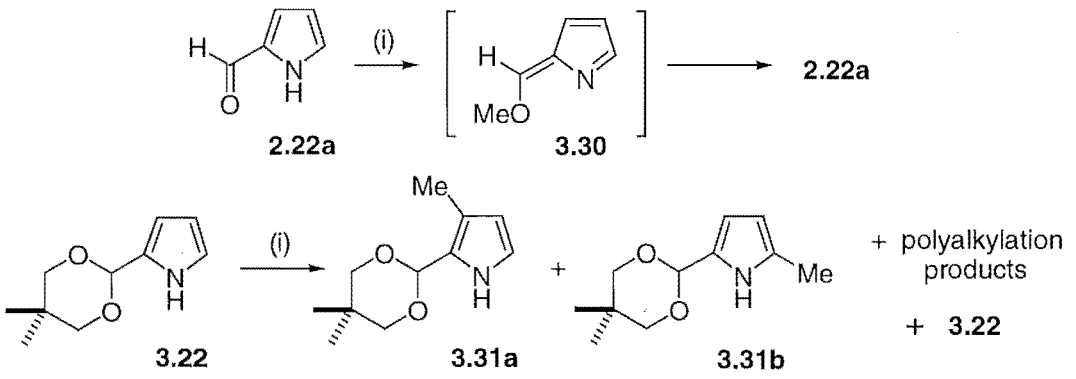
A similar reaction of *N*-mesyl-2-hydroxymethylpyrrole **2.25b** was then performed using methanesulfonic anhydride and Hünig's base in $CDCl_3$ at –20 °C. A $^1H$ NMR spectrum of the reaction mixture immediately after addition showed that the majority of the hydroxymethylpyrrole **2.25b** had been converted into the *O*-mesylate **3.26**, with only a trace (~5%) of **2.25b** still remaining. Subsequent spectral analysis found that the *O*-mesylate **3.26** was stable up to 0 °C, after which substantial conversion back to the hydroxymethylpyrrole **2.25b** was observed to occur.

## *3.3. Synthesis of dipyrromethanes using pyrrolylmagnesium derivatives*

Simple *N*-magnesio derivatives of pyrrole are reported to react preferentially with alkylating agents at carbon to produce a mixture of 2(α)- and 3(β)-alkylpyrroles, in which the 2-isomer usually predominates.[12] This is in contrast to the *N*-pyrrolyl alkali metal salts which react preferentially at nitrogen to give *N*-alkylpyrroles (see Chapter 2.1 for further discussion). However, if the pyrrole ring already contains substituents, the alkylation pattern on the pyrrolylmagnesium derivative becomes more complex. Electron donating groups at the 2-position of the pyrrole ring preferentially enhance alkylation at the 3- and 5-positions, with the 5-position the more activated site for substitution due to the effect of the annular nitrogen.[13] Furthermore, it has been reported that bulky alkylating agents further enhance the formation of the 2,5-product over the 2,3-product, and also tend to inhibit polyalkylation products, presumably due to steric reasons.[14] In contrast, electron withdrawing groups at the 2-position of the pyrrole ring promote

substitution at the 4- and 5-positions. The ratio of these two isomers depends on the *–M* effect of the 2-substituent, with a larger *–M* effect giving a greater proportion of the 4-isomer.[15] In addition, it has also been suggested that the *C*-alkylation of pyrrolylmagnesium salts occurs via an $S_N2$ mechanism, since it was reported that reaction with (–)-2-bromobutane gave the corresponding 2- and 3-substituted pyrroles with inversion of configuration at the alkyl group.[14a]

The feasibility of preparing α-formyldipyrromethanes by way of alkylation of a suitable pyrrolylmagnesium derivative at the α-free position was initially investigated using a model system. In an initial trial experiment, pyrrole-2-carboxaldehyde **2.22a** was deprotonated with methylmagnesium iodide to give the corresponding *N*-magnesio salt which precipitated from the reaction mixture. Excess methyl iodide was added and after stirring for 3 hours a homogeneous solution was obtained. However, subsequent work up of this solution gave recovered starting material **2.22a** only. It was possible that methylation had taken place on the formyl oxygen, rather than on carbon of the pyrrole, to give **3.30**, which had then been hydrolysed to **2.22a** on work up (Scheme 3.10). However, this seems unlikely as magnesium alkoxides are poor nucleophiles and methyl iodide is a soft electrophile. Therefore, at this stage it remains uncertain why only starting material was isolated. It was then postulated that protection of the formyl group, such as by its conversion to the acetal **3.22**, might eliminate this above complication and allow alkylation to proceed on carbon.




**Scheme 3.10.** *Reagents and conditions:* (i) MeMgI, THF, rt then MeI then $H_2O$.

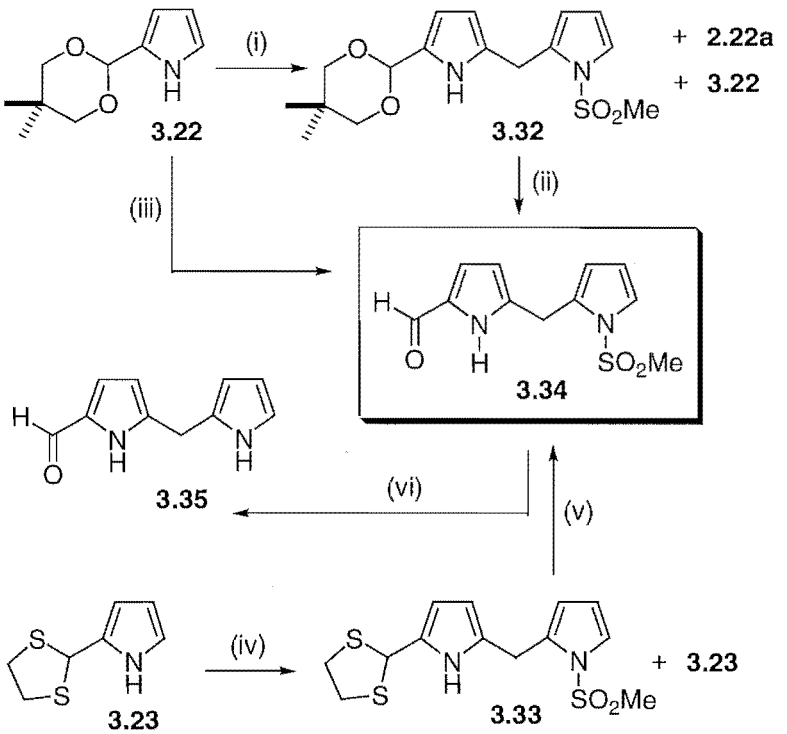
Indeed, reaction of **3.22** with methylmagnesium iodide, followed by the addition of an excess of methyl iodide gave an inseparable mixture of *C*-alkylated pyrroles and starting material **2.22a** in an approximate ratio of 2 : 5 by $^1$H NMR spectroscopy (Scheme 3.10). No attempt was made to fully characterise these alkylation products. However, on the basis of previous work on the alkylation of pyrrolylmagnesium salts,[14b] it was reasoned that the product mixture would consist predominantly of the 2,3- and 2,5-disubstituted products **3.31a** and **3.31b**, respectively, along with various other polyalkylation products.

Having established that we were able to effect *C*-alkylation of the formyl-protected pyrrole under these conditions, we turned our attention to the coupling of **3.22** with the chloromethylpyrrole **3.28** (Scheme 3.11) bearing an *N*-methanesulfonyl group to deactivate the pyrrole ring, and hence further favour an $S_N2$ substitution mechanism. As discussed in Chapter 2.1, hydroxymethyl- and chloromethylpyrroles lacking an *N*-mesyl or related group are reactive and labile. In contrast, the chloromethylpyrrole **3.28** and its synthetic precursor **2.25b** were stable compounds. The effect of an electron withdrawing group on the nitrogen of a chloromethylpyrrole was shown by determining the structure of the *N*-*p*-toluenesulfonyl derivative **3.29** by X-ray analysis (see Figure 3.1 and Section 3.6 for a discussion).

The *N*-mesylchloromethylpyrrole **3.28** was reacted with a 3.3 molar excess of the *N*-magnesio salt[16] derived from **3.22** and methylmagnesium iodide (Scheme 3.11). It has been reported that polyalkylation of the pyrrolylmagnesium species is an important reaction when equivalent quantities of alkylating agents are used,[16,17] therefore an excess of the *N*-magnesio salt derived from **3.22** was employed in an attempt to minimise such polyalkylation. Work up with aqueous acetic acid gave the doubly protected dipyrromethane **3.32** in admixture with pyrrole-2-carboxaldehyde **2.22a**. The coupling proceeded exclusively at the α-position to give the desired 2,5-disubstituted product, with none of the alternative 2,3- or polysubstituted products evident by $^1$H NMR spectroscopy. Deprotection of the formyl group was then achieved by treating the above sample of **3.32** with pyridinium tosylate in 50% aqueous acetone, to give the α-formyldipyrromethane

**3.34** in 60% overall yield for the 2 steps, which was fully characterised. An identical reaction of the *N*-magnesio salt of **3.22** with the chloromethylpyrrole **3.28**, followed by the addition of aqueous hydrochloric acid gave **3.34** directly in a yield of 63% (Scheme 3.11).

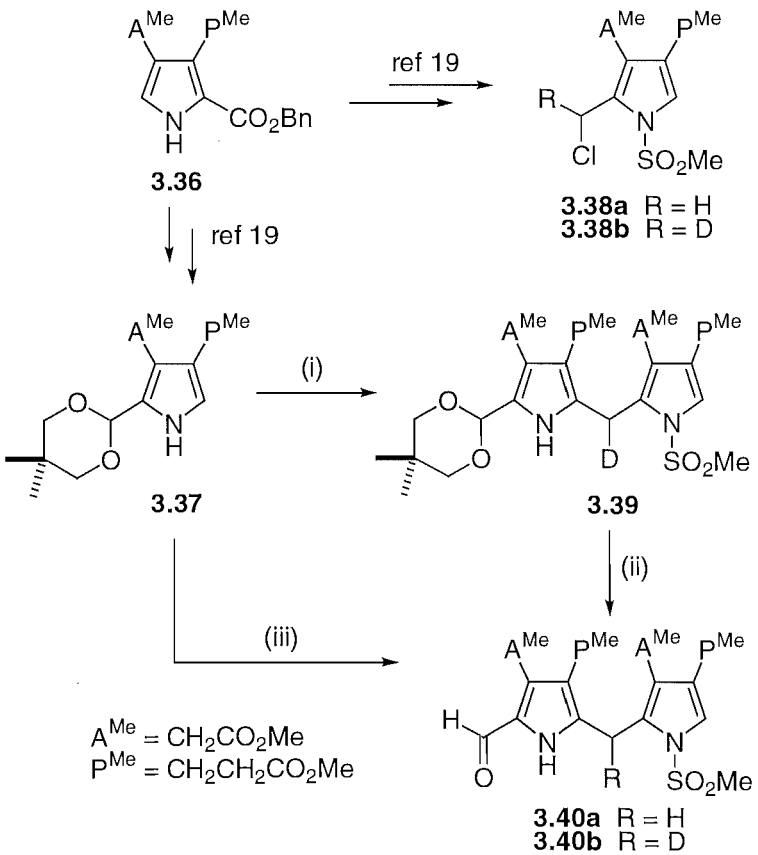
The thioacetal **3.23** was similarly treated with methylmagnesium iodide, and the resulting *N*-magnesio salt was coupled with the chloromethylpyrrole **3.28** to give the protected dipyrromethane **3.33** (Scheme 3.11). Again, no other alkylation isomers were evident by $^1$H NMR spectroscopy. Removal of the dithiolane protecting group from **3.33** was then achieved using mercuric chloride and calcium carbonate in 80% aqueous acetonitrile to give **3.34** in an overall yield of 40% for the two steps.




**Scheme 3.11.** *Reagents and conditions*: (i) MeMgI, –10 °C to rt then **3.28,** –10 °C to rt then 10% aq. AcOH; (ii) PPTS, acetone-$H_2O$ (1:1), reflux (60% for two steps); (iii) MeMgI, –10 °C to rt then **3.28**, –10 °C to rt then 10% aq. HCl (63%); (iv) MeMgI, –10 °C to rt then **3.28**, –10 °C to rt then aq. $NH_4Cl$ (50%); (v) $HgCl_2$, $CaCO_3$, MeCN-$H_2O$ (1:4) (83%); (vi) NaOH, MeOH-$H_2O$, reflux (85%).

Finally, removal of the *N*-methanesulfonyl deactivating group from **3.34** was accomplished by heating **3.34** with aqueous sodium hydroxide at reflux, to give **3.35** in 85% yield. The α-formyldipyrromethane **3.35** has previously been prepared by Clezy *et al.* in 59% yield by formylation of the 2,2'-dipyrromethane **3.19** (see Scheme 3.6).[18]

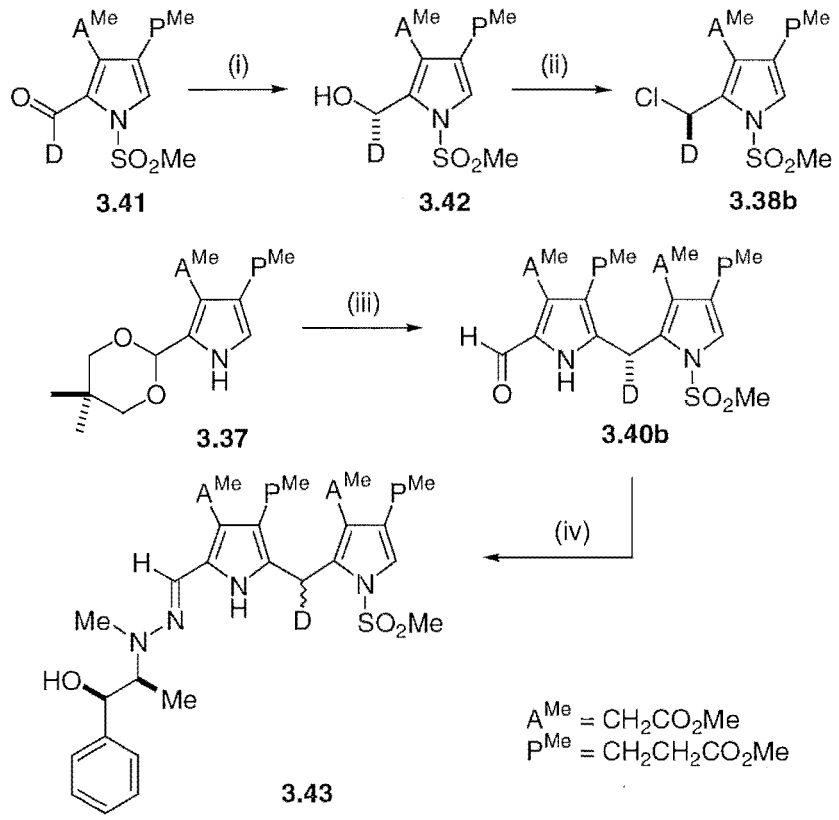
This pyrrolylmagnesium-based synthetic approach to dipyrromethanes was further extended, in work carried out in collaboration with Professor Sir Alan Battersby, by the preparation of **3.40a** and its deuterated analogue **3.40b** (Scheme 3.12). These dipyrromethanes are useful precursors to biologically important bilanes, as discussed in Section 3.1, with the deuterium-label in **3.40b** providing a potential marker that would enable the study of some of the key stereochemical issues associated with the biosynthesis of these compounds. The key starting materials **3.37** and **3.38**, that were




**Scheme 3.12.** *Reagents and conditions:* (i) MeMgI, THF, –10 °C then **3.38b**, –10 °C to rt then aq. AcOH (70%); (ii) dilute aq. HCl; (iii) MeMgI, THF, –10 °C then **3.38a**, –10 °C to rt then dilute aq. HCl (**3.40a**, 75%).

used in the synthesis of compounds **3.40**, were all prepared from a common pyrrole precursor **3.36**, in a number of steps, as recently detailed.[19] Next, reaction of the pyrrolylmagnesium salt derived from **3.37** with the *N*-mesylchloromethylpyrrole **3.38a**, followed by work up with aqueous hydrochloric acid, gave the α-formyldipyrromethane **3.40a** in 75% yield (Scheme 3.12). An analogous sequence using the racemic deuterated chloromethylpyrrole **3.38b**, but worked up with aqueous acetic acid, gave the doubly protected dipyrromethane **3.39** in 70% yield. Subsequent hydrolysis of **3.39** with aqueous hydrochloric acid gave the corresponding deuterium-labelled α-formyldipyrromethane **3.40b**.

Having successfully completed the synthesis of racemic deuterium-labelled dipyrromethane **3.40b**, work was then focussed on preparing a sample of **3.40b** that was stereoselectively labelled at the interpyrrolic methylene position. In order to accomplish this, a sample of optically active *N*-mesylhydroxymethylpyrrole **3.42** was prepared via the stereoselective reduction of the corresponding deuterated *N*-mesylformylpyrrole **3.41** using *R*-Alpine borane® (Scheme 3.13). We had shown in previous work that the reduction of a deuterated *N*-mesylformylpyrrole with *R*-Alpine borane® gave the corresponding hydroxymethylpyrrole with an excess of the *S*-isomer over the *R*-isomer of ~15:1 (see Chapter 2.3). Therefore, it was reasoned that the *R*-Alpine borane® reduction of the *N*-mesylformylpyrrole **3.41** would also have given the *S*-isomer of **3.42** in an excess of ~15:1. The chiral *N*-mesylhydroxymethylpyrrole **3.42** was then converted into the corresponding chloromethylpyrrole **3.38b** by treatment with methanesulfonyl chloride and Hünig's base. This chloromethyl formation was anticipated to proceed with inversion of configuration at the deuterated methylene centre of **3.42**, to give an excess of the *R*-isomer of **3.38b**. Subsequent coupling of this chloromethylpyrrole with the pyrrolylmagnesium salt derived from **3.37**, followed by hydrolysis under acidic conditions, gave the deuterium-labelled dipyrromethane **3.40b** in 66% yield (Scheme 3.13). On the basis of the work described in Chapter 2 it was hoped that this coupling would proceed under $S_N2$ conditions. Coupling under such conditions would result in an




**Scheme 3.13.** *Reagents and conditions:* (i) *R*-Alpine borane®, THF, rt (84%); (ii) Hünig's base, $MeSO_2Cl$ then 10% aq. HCl; (iii) MeMgI, THF, –10 °C then **3.38b**, –10 °C to rt then dilute aq. HCl (66%); (iv) *N*-amino-*l*-ephedrine, PhH, 4 Å sieves, reflux.

inversion of configuration at the deuterated methylene centre of **3.38b** to give an excess of the *S*-isomer of the dipyrromethane **3.40b**. To analyse the configuration at the deuterated methylene centre of this dipyrromethane sample, the α-formyl group of **3.40b** was coupled with the chiral amine, *N*-amino-*l*-ephedrine, to give the labelled hydrazone **3.43** (Scheme 3.13). As a consequence of this hydrazone formation, the interpyrrolic methylene protons of **3.43** are made diastereotopic, and are thus potentially resolvable by $^1$H NMR spectroscopy.

The ratio of diastereomers at the deuterated methylene centre of **3.43** was determined to be ~1:1 by integration of the methylene proton resonances at δ 3.89 and 3.97. Consequently, this indicated that the precursor dipyrromethane **3.40b** was racemic. This result suggested that either the coupling had not occurred under $S_N2$ conditions, or

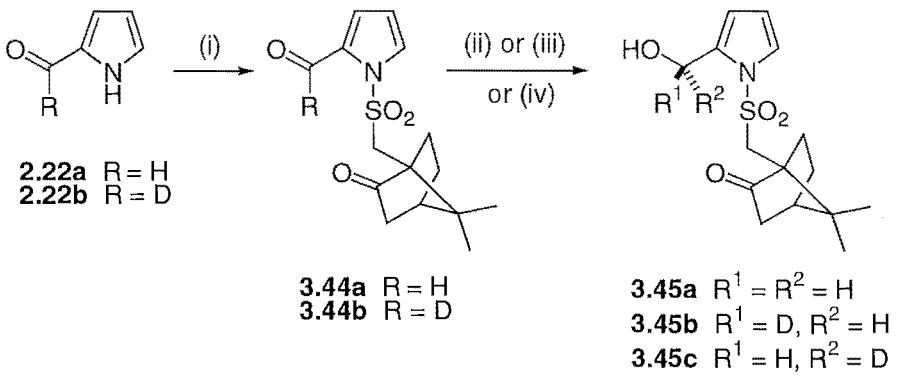
that the starting deuterium-labelled chloromethylpyrrole **3.38b** was racemic. Of these two possibilities, we felt that the latter explanation was the most likely cause for the observed loss of stereochemical integrity at the deuterated methylene centre during the synthetic sequence. This was in light of our earlier work, discussed in Chapter 2.4, which found that a considerable loss of configurational purity was obtained when a chiral *N*-mesylhydroxymethylpyrrole was reacted with a nucleophile under $S_N2$ conditions. This loss of stereochemical integrity was attributed to azafulvenium formation during the reaction sequence. Therefore, in the above attempted synthesis of a chiral sample of the deuterium-labelled dipyrromethane **3.40b**, where two separate $S_N2$ reactions were employed (*ie* for both chloromethylpyrrole formation and in the coupling procedure), there was considerable scope for a similar loss of stereochemical integrity due to this azafulvenium formation. Consequently, the *N*-mesyl group may therefore not be the best substituent to use when attempting the synthesis of a chiral deuterium-labelled dipyrromethane. Instead, a better substituent to employ on the pyrrole nitrogen in such a synthesis, so as to minimise this loss of stereochemical integrity, would be a triflyl group. An *N*-triflyl group has previously been found to be the optimal group on pyrrole for suppressing azafulvenium formation, due to its greater deactivating ability (see Chapter 2.4 for a discussion).

## *3.4. Investigation into the stereochemical course of chloromethyl- and* O*-mesylpyrrole formation*

The stereochemical course of chloromethylpyrrole formation was investigated in order to determine whether a chiral deuterium-labelled chloromethylpyrrole could be prepared under the conditions described for the formation of **3.38b**. We had established in previous work, discussed in Chapter 2.3, that deuterated hydroxymethylpyrroles could be prepared with a high degree of stereochemical purity through the use of a suitable

deactivating substituent on nitrogen. The analysis of the stereochemistry at the deuterated methylene centre of these hydroxymethylpyrroles was achieved by the attachment of a chiral derivatising agent or 'handle', namely the (1*S*,4*R*)-(–)-camphanate group, onto the hydroxymethyl position. However, such an approach to analyse the stereochemistry of deuterated chloromethylpyrroles is not possible due to the lack of a suitable functional group onto which to attach the required chirality. To circumvent this problem, we decided to incorporate the chiral handle into the nitrogen deactivating substituent. In this way it was envisaged that the stereochemical course of the hydroxymethyl- to chloromethylpyrrole transformation could be conveniently monitored by $^1$H NMR spectroscopy. The group that was selected to achieve this aim was (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride, a chiral derivatising agent which had previously been employed for the resolution of chiral amines.[20] This *N*-substituent, as an alkylsulfonyl group, would be expected to have a deactivating ability on the pyrrole ring comparable to that observed for the previously investigated methanesulfonyl group.

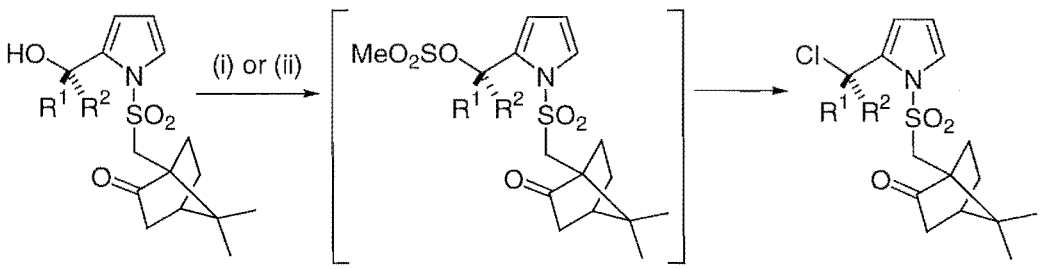
The key deuterium-labelled *N*-camphorsulfonyl hydroxymethylpyrroles **3.45b** and **3.45c** were prepared by treating the labelled formylpyrrole **2.22b** with sodium hydride, and reacting the resultant pyrrolyl anion with (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride to




**Scheme 3.14.** *Reagents and conditions:* (i) NaH, THF then (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride (**3.44a**, 95%), (**3.44b**, 99%); (ii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (**3.45a**, 88%); (iii) *S*-Alpine borane®, THF, rt (**3.45b:3.45c**, 19:1, 70%); (iv) *R*-Alpine borane®, THF, rt (**3.45b:3.45c**, 1:19, 76%).

give **3.44b** in 99% yield. This was then followed by the stereoselective reduction of the deuterated formyl group with either *S*- or *R*-Alpine borane®, to give mixtures of **3.45b** and **3.45c** in yields of 70% and 76%, respectively (Scheme 3.14). The ratios of **3.45b** and **3.45c** obtained from these *S*- and *R*-Alpine borane® reductions were determined to be 19:1 and 1:19, respectively, by integration of the corresponding pyrrole-CHD singlet resonances at δ 4.72 and 4.87 (see first column of spectra, Table 3.2). The unlabelled analogue **3.45a**, which was used as a reference compound in the subsequent analysis, was similarly prepared by N-substitution of pyrrole-2-carboxaldehyde **2.22a** with (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride, to give **3.44a** in 95% yield, followed by zinc borohydride reduction of the formyl group in 88% yield.

Next, we turned our attention to studying the stereochemical course of chloromethylpyrrole formation by $^1$H NMR spectroscopy. In the ensuing reactions, the unlabelled **3.47a** and deuterium-labelled chloromethylpyrroles **3.47b** and **3.47c** were observed to form from the corresponding mixtures of unlabelled **3.45a** and deuterated hydroxymethylpyrroles **3.45b** and **3.45c**, of known configurational purity, and Hünig's base in $CDCl_3$ at 0 °C, upon treatment with methanesulfonyl chloride [see Scheme 3.15, reaction conditions (i) and second column of spectra, Table 3.2]. Subsequent work up of these mixtures then gave the chloromethylpyrroles **3.47a**, **3.47b** and **3.47c** as the reaction products (see third column of spectra, Table 3.2).




**3.45a** $R^1 = R^2 = H$
**3.45b** $R^1 = D$, $R^2 = H$
**3.45c** $R^1 = H$, $R^2 = D$

**3.46a** $R^1 = R^2 = H$
**3.46b** $R^1 = D$, $R^2 = H$
**3.46c** $R^1 = H$, $R^2 = D$

**3.47a** $R^1 = R^2 = H$
**3.47b** $R^1 = D$, $R^2 = H$
**3.47c** $R^1 = H$, $R^2 = D$

**Scheme 3.15.** *Reagents and conditions:* (i) Hünig's base, $MeSO_2Cl$ then 10% aq. HCl; (ii) Hünig's base, $(MeSO_2)_2O$, $CDCl_3$, –20 °C.

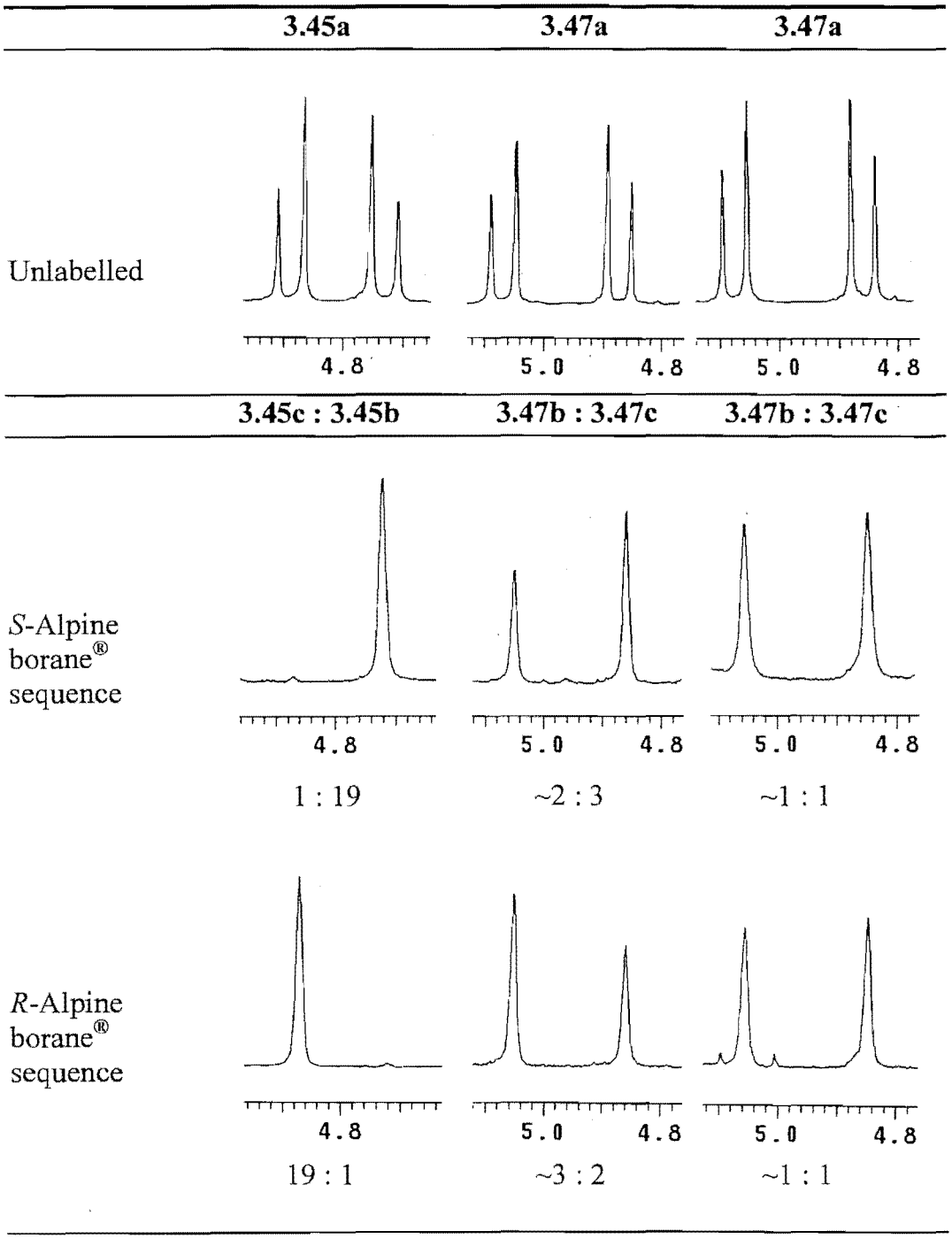



**Table 3.2.** $^1$H NMR resonances ($CDCl_3$) for the pyrrole *exo*-methylene group of **3.45** and the reaction product **3.47**. In the spectra of **3.45b-c**, coupling of the signals with the OH proton were removed by homonuclear decoupling.

The key point to note from the results shown in Table 3.2 is that compared to the configurational purity of the starting deuterated hydroxymethylpyrroles **3.45b** and **3.45c** (19:1), only a small excess of the corresponding chloromethylpyrrole isomer (~3:2), resulting from net inversion of the stereochemistry at the *exo*-methylene position, was observed to form during the chlorination reaction. This isomer ratio of ~3:2 for the respective chloromethylpyrrole products was, however, in agreement with the isomer ratio of ~3:2 previously obtained from the Mitsunobu reaction of chiral *N*-mesylhydroxymethylpyrroles with (1*S*,4*R*)-(–)-camphanic acid under $S_N2$ conditions (see Chapter 2.4). Therefore, as predicted, this demonstrates that the two alkylsulfonyl groups, camphorsulfonyl and methanesulfonyl, have analogous deactivating abilities on the pyrrole ring, and so both suppress azafulvenium formation to similar extents. A second point to note is that upon work up of the reaction mixtures, ~1:1 mixtures of the chloromethylpyrroles **3.45b** and **3.45c** were obtained (rows 2 and 3 of Table 3.2). This indicates that further azafulvenium formation probably occurs under the conditions used in the work up procedure, resulting in the total scrambling of the deuterium-label at the *exo*-methylene position. This observed scrambling of a chiral deuterium-label during chloromethylpyrrole formation, therefore confirms our earlier proposition that this was the cause of the racemisation encountered in our attempted synthesis of a chiral sample of the dipyrromethane **3.40b** (see Scheme 3.13). A final point to note from the results shown in Table 3.2 is that in the case of the *N*-camphorsulfonyl hydroxymethylpyrroles, the methylene resonance is upfield for the *R*-isomer (*eg* **3.45b**) relative to the *S*-isomer (*eg* **3.45c**). This configurational assignment was based on the work of Midland *et al.*[21] which had shown that reduction of an aldehyde by *S*- or *R*-Alpine borane® gives the *R*- or *S*-alcohol, respectively, with a high degree of enantiomeric purity. In the case of the *N*-camphorsulfonyl chloromethylpyrroles **3.47b** and **3.47c**, however, the observed methylene resonances were reversed with respect to **3.45b** and **3.45c**, with the methylene resonance upfield for the *S*-isomer relative to the *R*-isomer. The assignment of the configurations of the chloromethylpyrroles **3.47b** and **3.47c** were based on the

assumption that the chlorination reaction proceeds with inversion of configuration at the deuterated methylene centre.

Having established that chloromethylpyrrole formation proceeded with scrambling of the deuterium-label at the *exo*-methylene position, we turned our attention to investigating the stereochemical course of *O*-mesylate formation in order to ascertain whether we could prepare chiral *O*-mesylates. Such chiral *O*-mesylates might prove suitable for coupling in the stereoselective synthesis of labelled dipyrromethanes. Consequently, the conversion of labelled *N*-camphorsulfonylpyrroles **3.45b** and **3.45c** to the *O*-mesylates **3.46b** and **3.46c** was followed by low temperature $^1$H NMR spectroscopy [see Scheme 3.15, reaction conditions (ii)]. Spectral analysis showed that upon addition of methanesulfonic anhydride to a mixture of **3.45b** and **3.45c** (19:1 by $^1$H NMR) and Hünig's base in $CDCl_3$ at –20 °C, the corresponding *O*-mesylates **3.46b** and **3.46c** were observed to form, albeit in a lower isomer ratio of ~4:1. A $^1$H NMR spectrum of the reaction mixture after 30 min showed a ~1:1 ratio of the two isomers, indicating the total scrambling of the deuterium-label at the *exo*-methylene position. This loss of stereochemical integrity at the deuterated methylene centre was again attributed to azafulvenium formation during the reaction sequence. The epimeric ratio of the *O*-mesylates **3.46c** and **3.46b** was determined by integration of the pyrrole-CHD singlet resonances at δ 5.39 and 5.45, respectively. As in the case of the chloromethylpyrroles **3.47b** and **3.47c** described previously, the methylene resonance of the *O*-mesylates **3.46b** and **3.46c** was upfield for the *S*-isomer relative to the *R*-isomer. These configurational assignments were based on the assumption that the initial *O*-mesylation reaction proceeds with retention of configuration at the deuterated methylene centre. Finally, in the complementary experiment, the mesylation reaction carried out with the hydroxymethylpyrroles **3.45b** and **3.45c** (1:19 by $^1$H NMR) also showed scrambling of the deuterium-label. The *O*-mesylates **3.46b** and **3.46c** were initially observed to form in a isomer ratio of ~1:4, however, a $^1$H NMR spectrum of the reaction mixture after 90 min

showed only a ~1:1 ratio of the two isomers. This scrambling of the deuterium-label during the reaction sequence was again attributed to azafulvenium formation.

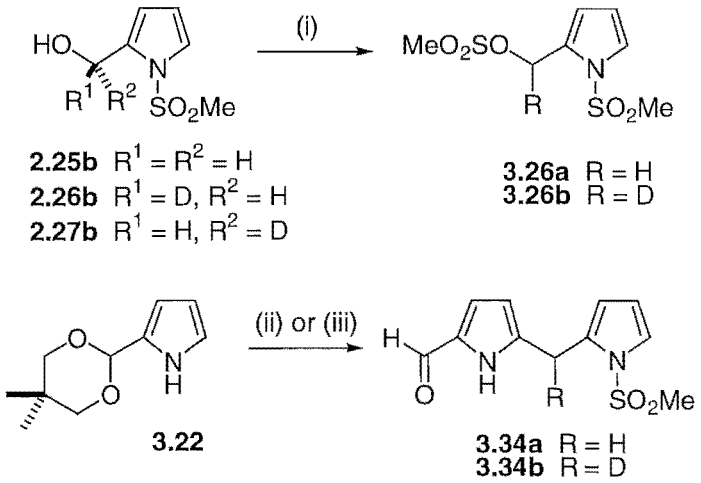
These results demonstrate that the scrambling of the deuterium-label at the *exo*-methylene position of **3.45b** and **3.45c** occurs readily during *O*-mesylate formation, even at low temperature. This fact, therefore, greatly restricts any attempt to prepare chiral deuterium-labelled dipyrromethanes of the type **3.40b** using the synthetic methodology detailed in Section 3.3. This is due to the propensity of the chiral starting material to undergo racemisation under the reaction conditions used to activate the leaving group towards nucleophilic displacement. It is important to note again, however, that this investigation was carried out using a camphorsulfonyl group to deactivate the pyrrole, due to the need for a chiral handle to allow discrimination of the stereochemistry at the deuterated methylene centre. As discussed previously, this would not be the best substituent for deactivating the pyrrole ring, and hence suppressing azafulvenium formation. Instead, the optimal substituent to employ on the pyrrole nitrogen during *O*-mesylate formation in order to minimise this observed racemisation would be a triflyl group due to its greater deactivating ability on the pyrrole ring.

## *3.5. Attempted preparation of chiral deuterium-labelled dipyrromethane*

From the above low temperature mesylation studies, it was therefore concluded that for the best chance of preparing a chiral deuterium-labelled dipyrromethane, an *O*-mesylate would need to be generated from the corresponding chiral hydroxymethylpyrrole at low temperature, and then be immediately coupled with the pyrrolylmagnesium salt, also at low temperature. In this way, it was hoped that the degree of scrambling of the deuterium-label in the resulting dipyrromethane could be minimised, by reacting the *O*-mesylate before it underwent an appreciable amount of epimerisation at the methylene centre. In this study, the *N*-mesyl group was again used to

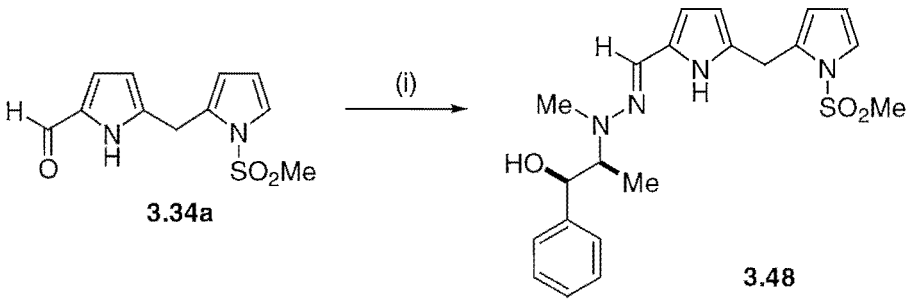
deactivate the pyrrole ring in preference to the more deactivating *N*-triflyl group, as this group would allow a direct comparison with our previously described synthesis of the *N*-mesyldipyrromethane **3.34a**, which utilised the coupling of a pyrrolylmagnesium salt with a chloromethylpyrrole (see Section 3.3).

In the ensuing reaction, the bismesylate **3.26a**, generated at –40 °C from **2.25b**, was coupled with the pyrrolylmagnesium salt derived from **3.22**. Subsequent work up with aqueous hydrochloric acid then gave the dipyrromethane **3.34a** in 53% yield (Scheme 3.16). The corresponding sequence was repeated using the bismesylate **3.26b**, generated at –40 °C from a mixture of **2.26b** and **2.27b** (~1:15 by camphanate analysis as described in Chapter 2.3). By analogy to the low temperature *O*-mesylation studies described in Section 3.4, the bismesylate **3.26b** formed was assumed to have a configurational purity of ~4:1 at the deuterated methylene centre. Subsequent coupling of **3.26b** with the pyrrolylmagnesium salt derived from **3.22**, followed by hydrolysis under acidic conditions, gave the deuterium-labelled dipyrromethane **3.34b** in 54% yield (Scheme 3.16).




**Scheme 3.16.** *Reagents and conditions:* (i) Hünig's base, $(MeSO_2)_2O$, $CH_2Cl_2$, –40 °C; (ii) MeMgI, –10 °C to rt then **3.26a**, –40 °C to rt then 10% aq. HCl (**3.34a**, 53%); (iii) MeMgI, –10 °C to rt then **3.26b**, –40 °C to rt then 10% aq. HCl (**3.34b**, 54%).

Attempts to investigate the stereochemical integrity at the interpyrrolic methylene centre of **3.34** proved problematic. Preparation of the chiral hydrazone **3.48** of the unlabelled dipyrromethane **3.34a** (Scheme 3.17) and subsequent $^1$H NMR spectral analysis failed to differentiate the interpyrrolic methylene protons. This was in contrast to the previously prepared hydrazone **3.43**, in which the interpyrrolic methylene protons were readily resolved by $^1$H NMR spectroscopy (see earlier for a discussion). Various NMR solvents, including $CDCl_3$, $C_6D_6$, $CD_3CN$, pyridine-$d_5$, acetone-$d_6$, and $CD_3OD$, were unsuccessfully utilised in an attempt to circumvent this problem. However, the $^1$H NMR spectra of **3.48** in all these solvents failed to show any discrimination of the interpyrrolic methylene protons.




**Scheme 3.17.** *Reagents and conditions:* (i) *N*-amino-*l*-ephedrine, PhH, 4 Å sieves, reflux (62%).

Consequently, other methods were tried in an attempt to differentiate the interpyrrolic methylene protons of our dipyrromethanes. These included the attempted N-substitution of **3.34a**, and also of **3.35**, with the previously described chiral derivatising agent, (1*S*,4*R*)-(+)-camphorsulfonyl chloride. However, all attempts at N-substitution of **3.34a** and **3.35** using this chiral handle were unsuccessful. An attempt was also made to resolve the different enantiomers of **3.34b** by deuterium NMR spectroscopy in the chiral liquid crystalline solvent poly-(γ-benzyl-*L*-glutamate) (PBLG)/dichloromethane. This method has previously been reported to be an efficient method for the resolution of chirality at the deuterated centre of various compounds.[22] Examples of deuterated

compounds which have been resolved using this method include alcohols, acids, esters, ethers, epoxides, halides and tosylates. The resolution of two unsaturated hydrocarbons by this method has also been reported,[22b] although the authors of this work questioned the applicability of this technique to resolve other hydrocarbons. Consequently, a sample of **3.34b** was dissolved in the chiral liquid crystalline solvent and analysed by deuterium NMR spectroscopy. However, the subsequent spectral analysis failed to show any resolution of the enantiomers of **3.34b**.

Consequently, due to an inability to discriminate the interpyrrolic methylene protons of the product dipyrromethanes by the above mentioned spectroscopic techniques, we have been unable to ascertain whether we have prepared a chiral sample of the deuterium-labelled dipyrromethane **3.34b** using our modified coupling procedure. We have, however, developed a new synthetic procedure for the preparation of unlabelled and labelled dipyrromethanes, which are themselves important intermediates to biologically important bilanes.

## *3.6. X-Ray structure determination of* N*-tosyl-2-chloromethylpyrrole 3.29*

The X-ray crystal structure of **3.29** was determined to show its preferred conformation in the solid state, and also to measure the effect of an electron withdrawing *N*-substituent (*eg* tosyl) on the aromaticity of the pyrrole ring. A search of the Cambridge Crystallographic Data Base revealed only a few structures of *N*-tosyl and *N*-mesyl pyrroles had been previously reported.[23]

A perspective drawing of **3.29**, with atomic labelling, is presented in Figure 3.1. The first point to note regarding this structure is that the N(1)-C(2) and N(1)-C(5) bond lengths are relatively long [1.412(3) and 1.392(3) Å, respectively] as compared to pyrroles without an electron withdrawing substituent on nitrogen.[24] In addition, the C(2)-C(3) and C(4)-C(5) bond lengths are relatively short at 1.353(4) and 1.342(4) Å,

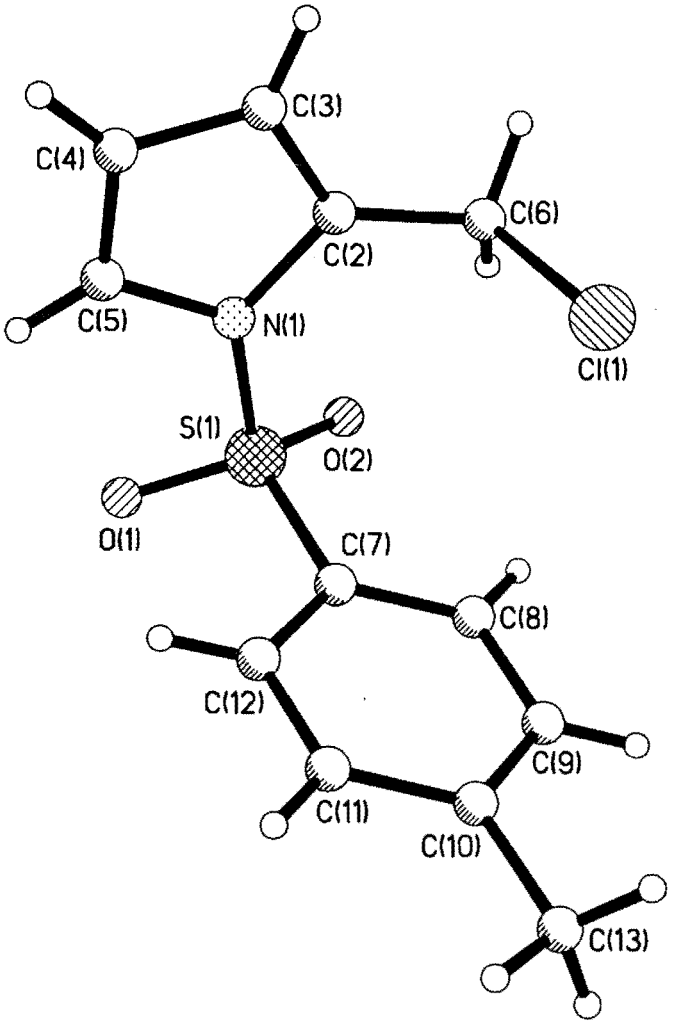



**Figure 3.1.** ORTEP diagram of **3.29** showing the crystallographic numbering scheme.

respectively. These observations show that the aromaticity of the pyrrole ring is significantly reduced by introduction of an electron withdrawing group (*eg* tosyl) on nitrogen. The result is that derivatives of the type **3.28** and **3.29** are stable entities which are suitable for controlled reactions with nucleophiles. This was demonstrated in the present study, where the stability of the *N*-mesylated chloromethylpyrroles **3.28** and **3.38**, was exploited through a controlled nucleophilic displacement reaction at the methylene position by an *N*-pyrrolylmagnesium salt, to give the dipyrromethanes **3.34** and **3.40**, respectively. A similar reaction without an electron withdrawing substituent on the

pyrrole nitrogen, would lead to the formation of a highly reactive azafulvene, which could then react in an uncontrolled manner (see Chapter 2.1 for a discussion).

The second point to note regarding the crystal structure of **3.29** is that the tosyl group adopts the expected tetrahedral geometry about S(1) [O(2)-S(1)-O(1) 120.7°, N(1)-S(1)-C(7) 105.4°, N(1)-S(1)-O(1) 104.2°, N(1)-S(1)-O(2) 106.9°, C(7)-S(1)-(O1) 109.3°, C(7)-S(1)-(O2) 109.2°]. The X-ray structures of related *N*-mesyl and *N*-tosyl pyrroles reveal that the sulfonyl group adopts a pseudo-staggered orientation with respect to the pyrrole ring[23c,24] where the S-methyl or aryl vector is almost perpendicular to the pyrrole ring plane. The structure of **3.29** reported here displays a significant deviation from this staggered geometry as depicted in Figure 3.2, left hand structure [C(7)-S(1)-N(1)-C(2) 78.9°, C(7)-S(1)-N(1)-C(5) –102.5°]. The equivalent conformation adopted by the S-pyrrole vector shows an even greater deviation from a staggered geometry as depicted in Figure 3.2, right hand structure [C(8)-C(7)-S(1)-N(1) 71.8°, C(12)-C(7)-S(1)-N(1) –108.2°]. Finally, the sum of the angles at nitrogen [C(2)-N(1)-S(1) 129.1°, C(2)-N(1)-C(5) 107.7°, C(5)-N(1)-S(1) 123.3°] is 360.1°, which is consistent with a planar nitrogen.

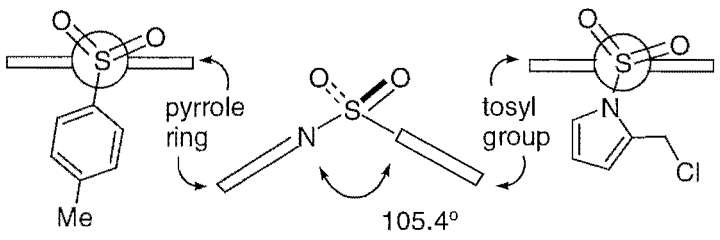



**Figure 3.2.** X-ray crystallographic conformation of **3.29**.

## 3.7. Conclusion and future work

In conclusion, we have developed a short, convenient and versatile synthetic route to dipyrromethanes which involves the coupling of an *N*-magnesio pyrrole salt (derived from either an oxygen acetal or a thioacetal of pyrrole-2-carboxaldehyde) with a ring-deactivated chloromethylpyrrole. The coupling occurs at the free α-position of the pyrrole nucleus to give the desired 2,5-disubstituted product, with none of the alternative 2,3- or polysubstituted coupling products being isolated. This reaction sequence has been extended to the preparation of dipyrromethanes containing a deuterium-label at the interpyrrolic methylene position. These compounds could serve as useful biological probes for studying the biosynthesis of natural porphyrins and related pigments. However, subsequent analysis of this labelled dipyrromethane, by its conversion into a chiral hydrazone, showed that the deuterium-label had been scrambled during the synthetic sequence. It was reasoned that this loss of stereochemical integrity was due to either the coupling reaction not occurring under $S_N2$ conditions, or the more likely cause, due to the starting deuterium-labelled chloromethylpyrrole being racemic. In order to establish whether or not this latter reason was indeed responsible for the scrambling of the deuterium-label, the stereochemical course of chloromethyl- and *O*-mesylpyrrole formation was investigated by $^1$H NMR spectroscopy. This work found that scrambling of a chiral deuterium-label occurred rapidly, even at low temperature, under the conditions used to activate the hydroxymethylpyrrole towards nucleophilic attack. A modified coupling procedure was then developed which was envisaged might minimise the scrambling of the deuterium-label during the dipyrromethane preparation. Unfortunately, however, all attempts to analyse the stereochemistry of the interpyrrolic methylene position of this dipyrromethane proved unsuccessful, and so the question of the effectiveness of our modified procedure in preparing chiral deuterium-labelled dipyrromethanes remains unanswered.

Future work could therefore entail repeating this modified coupling procedure using other, more highly substituted, pyrrole substrates, which have been shown previously to allow resolution of the interpyrrolic methylene position by $^1$H NMR spectroscopy upon hydrazone formation. This would allow the effectiveness of our modified coupling procedure in preparing chiral deuterated dipyrromethanes to be ascertained. Further work could also entail repeating this modified coupling procedure using the more highly deactivating triflyl group on the pyrrole nitrogen in order to minimise scrambling of the deuterium-label. In this way, the preparation of deuterated dipyrromethanes with a higher degree of enantiopurity at the interpyrrolic methylene position might be achieved.

Finally, the influence of an *N*-tosyl group on the aromaticity of the pyrrole ring was examined by the determination of the X-ray crystal structure of the *N*-tosyl-2-chloromethylpyrrole **3.29**.

## 3.8. References for chapter three


1 (a) Battersby, A. R.; Fookes, C. J. R.; Gustafson-Potter, K. E.; McDonald, E.; Matcham, G. W. T. *J. Chem. Soc., Perkin Trans. I.* **1982**, 2427; (b) Battersby, A. R.; Leeper, F. J. *Chem. Rev.* **1990**, *90*, 1261; (c) Scott, A. I. *Advances in Detailed Reaction Mechanisms: Mechanisms of Biological Importance.* Coxon, J. M. Ed. JAI Press: Connecticut, 1992, pp189-212.

2 For a recent review see: Blanche, F.; Cameron, B.; Crouzet, J.; Debussche, L.; Thibaut, D.; Vuilhorgne, M.; Leeper, F. J.; Battersby, A. R. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 383.

3 (a) Battersby, A. R.; Fookes, C. J. R.; Gustafson-Potter, K. E.; McDonald, E.; Matcham, G. W. J. *J. Chem. Soc., Perkin Trans. I.* **1982**, 2413; (b) Battersby, A. R.; Fookes, C. J. R.; Gustafson-Potter, K. E.; McDonald, E.; Matcham, G. W. J. *J. Chem. Soc., Perkin Trans. I.* **1982**, 2427.

4 (a) Jones, A. R.; Bean, G. P. Eds. *The Chemistry of Pyrroles.* Academic Press: London, 1977; (b) Katritzky, A. R.; Rees, C. W. Eds. *Comprehensive Heterocyclic Chemistry.* Pergamon Press: Oxford, 1984, Vol 4; (c) Tietze, L. F.; Kettschau, G.; Heitmann, K. *Synthesis* **1996**, 851.

5 Tarlton, E. J.; MacDonald, S. F.; Baltazzi, E. J. *J. Am. Chem. Soc.* **1960**, *82*, 4389.

6 Fischer, H.; Baumann, E.; Riedl, H. J. *Annalen*, **1929**, *475*, 205.

7 Chong, R.; Clezy, P. S.; Liepa, A. J.; Nichol, A. W. *Aust. J. Chem.* **1969**, *22*, 229.

8 (a) Clezy, P. S.; Nichol, A. W. *Aust. J. Chem.* **1965**, *11*, 1835; (b) Smith, K. M. *Quart. Rev.* **1971**, *25*, 31.

9 Schauder, J. –R.; Jendrezejewski, S.; Abell, A. D.; Hart, G. J.; Battersby, A. R.; *J. Chem. Soc., Chem. Commun.* **1987**, 436.

10 Loader, C. E.; Anderson, H. J. *Synthesis* **1978**, 295.

11 Wallace, D. M.; Leung, S. H.; Senge, M. O.; Smith, K. M. *J. Org. Chem.* **1993**, *58*, 7245.

12 (a) Jones, R. A. *Comprehensive Heterocyclic Chemistry.* Katritzky, A. R.; Rees, C. W. Eds. Pergamon Press: Oxford, 1984, Vol 4, pp235-240; (b) Anderson, H. J.; Loader, C. E. *Pyrroles.* Jones, R. A. Ed. John Wiley & Sons: New York, 1990, Vol 48, Part 1, pp427-428.

13 Jones, A. R.; Bean, G. P. Eds. *The Chemistry of Pyrroles.* Academic Press: London, 1977, pp192-195.

14 (a) Skell, P. S.; Bean, G. P. *J. Am. Chem. Soc.* **1962**, *84*, 4655; (b) Bean, G. P. *J. Org. Chem.* **1967**, *32*, 228.

15 Jones, R. A. *Comprehensive Heterocyclic Chemistry.* Katritzky, A. R.; Rees, C. W. Eds. Pergamon Press: Oxford, 1984, Vol 4, pp205-206.

16 Schloemer, G. C.; Greenhouse, R.; Muchowski, J. M. *J. Org. Chem.* **1994**, *59*, 5230.

17 Jones, A. R.; Bean, G. P. Eds. *The Chemistry of Pyrroles.* Academic Press: London, 1977, pp178-184.

18 Clezy, P. S.; Liepa, A. J.; Webb, N. W. *Aust. J. Chem.* **1972**, *25*, 1991.

19 Abell, A. D.; Nabbs, B. K.; Battersby, A. R. *J. Org. Chem.* **1998**, *63*, 8163.

20 (a) Dehmlow, E. V.; Westerheide, R. *Synthesis* **1992**, 947.; (b) Theodore, L. J.; Nelson, W. L. *J. Org. Chem.* **1987**, *52*, 1309; (c) Braun, H.; Felber, H.; Kresze, G.; Schmidtchen, F. P.; Prewo, R.; Vasella, A. *Liebigs Ann. Chem.*, **1993**, 261.

21 Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352.

22 (a) Meddour, A.; Atkinson, D.; Loewenstein, A.; Courtieu, J. *Chem. Eur. J.* **1998**, *4*, 1142; (b) Canet, I.; Courtieu, J.; Loewenstein, A.; Meddour, A.; Péchiné, J. M. *J. Am. Chem. Soc.* **1995**, *117*, 6520; (c) Meddour, A.; Canet, I.; Loewenstein, A.; Péchiné, J. M.; Courtieu, J. *J. Am. Chem. Soc.* **1994**, *116*, 9652.

[23] (a) Bennet, A. J.; Somayaji, V.; Brown, R. S.; Santarsiero, B. D. *J. Am. Chem. Soc.* **1991**, *113*, 7563; (b) Allen, F. H.; Battersby, A. R.; De Voss, J. J.; Doyle, M. J.; Raithby, P. R. *Acta Crystallogr., Sect. C* **1989**, *C45*, 692; (c) Beddoes, R. L.; Dalton, L.; Joule, J. A.; Mills, O. S.; Street, J. D.; Watt, C. I. F. *J. Chem. Soc., Perkin Trans. II.* **1986**, 787.

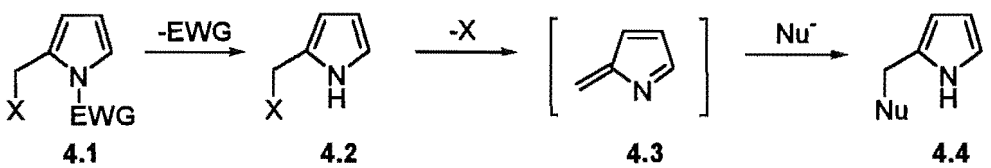
[24] Chadwick, D. J. *Pyrroles.* Jones, R. A. Ed. John Wiley & Sons: New York, 1990, Vol 48, Part 1, pp22-30.

# CHAPTER FOUR

# HYDROLYSIS STUDY OF DEUTERIUM-LABELLED *N*-ACYLHYDROXYMETHYLPYRROLES

## 4.1. Introduction

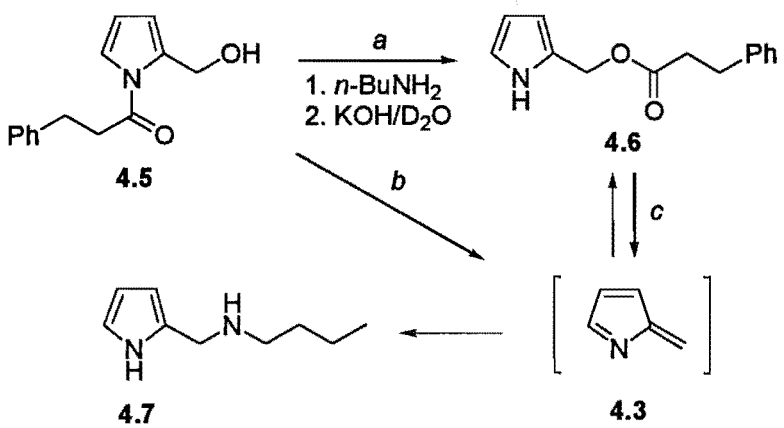
Much research has been directed towards the design and synthesis of amino acid analogues that release latent reactivity on the specific action of a target enzyme to give covalent inactivation.[1] As such, hydroxymethylpyrroles of general type **4.1** (X = OH), substituted with an electron withdrawing group (EWG) on nitrogen, have been developed, from work in our laboratories, as a new class of mechanism-based inhibitor of serine proteases (see Chapter 5 for further work in this area). However, for hydroxymethylpyrroles of type **4.1** to be useful as mechanism-based inhibitors, a key requirement is that the inherent reactivity of these compounds should only be released on demand (*ie* upon activation), and in a controlled manner. In the case of compounds of type **4.1**, this requirement is able to be achieved through either the chemical or enzymatic removal of the EWG (Scheme 4.1). The resulting non-deactivated pyrrole **4.2** can then readily undergo loss of the leaving group (X) to form a reactive azafulvene intermediate **4.3** – the chemical species responsible for the inherent (or latent) reactivity of this class of compound. This azafulvene species **4.3** can then be trapped by an external nucleophile ($Nu^-$) to give products of the type **4.4**. If this hydrolysis procedure occurs while **4.1** is bound to the target enzyme, the resulting azafulvene **4.3** could then be trapped by one of several suitable nucleophilic residues in the active site, resulting in covalent attachment and irreversible enzyme inhibition. Consequently, a key issue in the application of hydroxymethylpyrroles of type **4.1** as mechanism-based inhibitors of serine proteases is




**Scheme 4.1.** Azafulvene formation of 2-substituted pyrroles.

the mechanism by which this removal (or hydrolysis) of the EWG proceeds, thereby enabling the release of the latent reactivity.

Previous work in this area, using the *N*-hydrocinnamoyl hydroxymethylpyrrole **4.5**, had shown that during the chemical hydrolysis of this compound, using potassium hydroxide in the presence of *n*-butylamine (an external nucleophile), an *O*-acyl intermediate **4.6** was observed to form.[2] This was postulated to have occurred through either an initial *N*- to *O*-acyl transfer [Scheme 4.2, pathway (*a*)], or by simple hydrolysis of **4.5** followed by trapping of the azafulvene intermediate **4.3** with released hydrocinnamate [Scheme 4.2, pathway (*b*)]. Subsequently, the *O*-acylpyrrole **4.6** was converted into the aminomethylpyrrole **4.7** over a period of 90 min. This conversion was postulated to proceed via the nucleophilic attack of *n*-butylamine on the azafulvene intermediate **4.3**. This azafulvene **4.3** could have been generated from either the hydrolysis of **4.5** [Scheme 4.2, pathway (*b*)], via the loss of the *O*-acyl group from **4.6** [Scheme 4.2, pathway (*c*)], or by a combination of both pathways. This sequence also serves to model the proposed mechanism of action of compounds of type **4.5** as mechanism-based inhibitors of serine proteases – the potassium hydroxide mimics the protease catalysed deacylation and the external nucleophile mimics the final inactivation step.



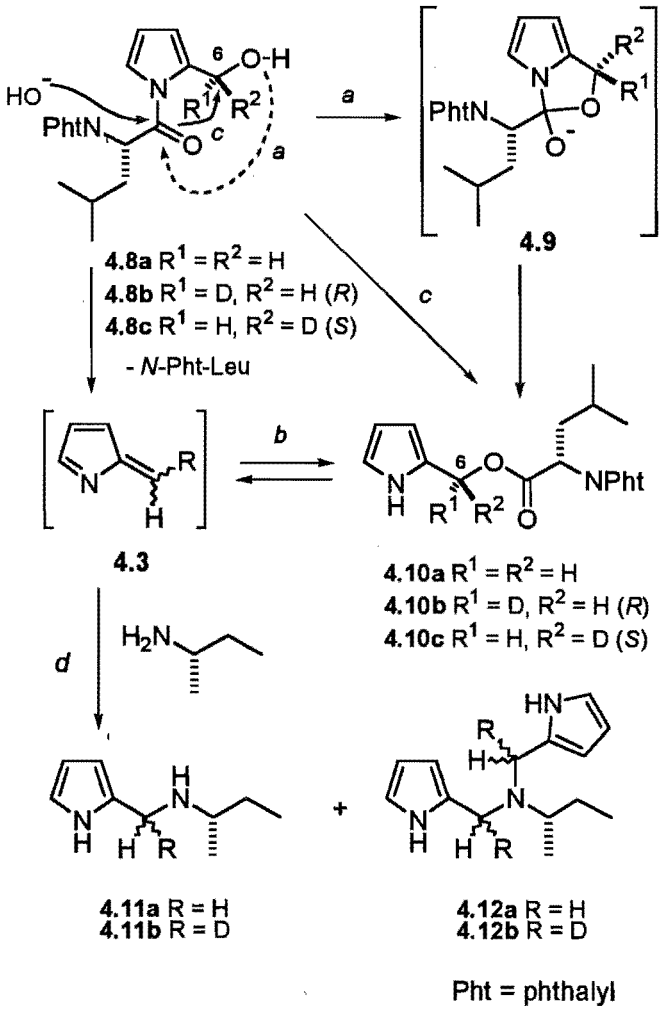

**Scheme 4.2.** Hydrolysis of **4.5** with KOH in the presence of *n*-$BuNH_2$.

Another important point to note from this study was the observation that the hydroxymethylpyrrole **4.5** did not react with *n*-butylamine over 48 hours in the absence of potassium hydroxide. This is in agreement with our previous work (see Chapters 2 and 3 for a discussion) which has shown that an electron withdrawing substituent on the pyrrole nitrogen, as in **4.1**, suppresses azafulvene formation and hence substitution at the *exo*-methylene position. This also provides evidence for the suitability of compounds of type **4.1** as mechanism-based inhibitors of serine proteases, as they fulfil the fundamental requirement that the inherent reactivity is only released on demand (*ie* upon removal of the EWG).

Despite the above preliminary work having been carried out on the hydrolysis of *N*-substituted hydroxymethylpyrroles, many of the issues concerning the exact mechanism of this process still remained unresolved, such as the mode of formation of the *O*-acyl intermediate and whether an azafulvene was the intermediate in the formation of the aminomethylpyrrole final product. We therefore set out to investigate these unresolved questions, and also to further develop a model system which would allow the mechanism of action of this class of compound as latent inhibitors of serine proteases to be determined.

To address these issues, work was centred on determining the stereochemical fate of a deuterium-label in the *R*- and *S*-[*methylene*-$d_1$]-*N*-(*N*-phthalyl-*L*-leucinyl) hydroxymethylpyrroles **4.8b** and **4.8c** upon potassium hydroxide catalysed hydrolysis in the presence of an external chiral nucleophile [*S*-(+)-*sec*-butylamine] (see Scheme 4.3). The *N*-acylhydroxymethylpyrrole **4.8** is an example of the general compound **4.1**, in which the electron withdrawing group is an *N*-protected amino acid. As has been previously proposed (see above for a discussion), the hydrolysis of compounds of type **4.8** (and therefore of compounds of type **4.1**) proceeds by an initial *N*- to *O*-acyl transfer, followed by the subsequent release of an azafulvene (the latent reactive species). In theory, the formation of the *O*-acylpyrrole **4.10** could occur via one of three distinct mechanisms, as detailed in Scheme 4.3. *O*-Acylpyrrole formation could occur by either

an intramolecular acyl transfer via the tetrahedral intermediate **4.9** [Scheme 4.3, pathway (*a*)], by the trapping of the azafulvene **4.3** with released *N*-phthalyl-*L*-leucine [Scheme 4.3, pathway (*b*)], or by an $S_N2$ like displacement [Scheme 4.3, pathway (*c*)]. Each of these pathways would have a different effect on the configuration of a deuterium-label present at the *exo*-methylene position of **4.8**. Thus, reaction via pathways (*a*), (*b*) and (*c*) would lead to the retention, epimerisation or inversion of configuration of a deuterium-label at the *exo*-methylene position, respectively. The azafulvene **4.3** formed on deacylation of **4.8** would then be trapped by an external nucleophile, either by *S*-(+)-*sec*-butylamine or subsequently by the pyrrole amine **4.11b**, to give the aminomethylpyrroles

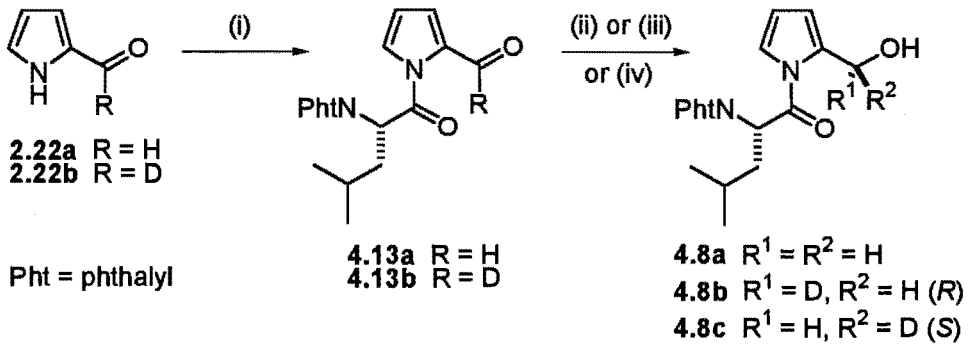



**Scheme 4.3.** Proposed reaction sequence to determine the mechanistic course of the hydrolysis reaction.

**4.11b** and **4.12b**, respectively [Scheme 4.3, pathway (*d*)]. Consequently, by using the stereoselectively deuterium-labelled *N*-acylhydroxymethylpyrroles **4.8b** and **4.8c**, and determining the position (or positions) of the deuterium-label in the *O*-acylpyrrole intermediates **4.10b** and **4.10c**, and in the aminomethylpyrrole end products **4.11b** and **4.12b** formed during the hydrolysis reaction by $^1$H NMR spectroscopy, the mechanistic course of the hydrolysis reaction for *N*-acylhydroxymethylpyrroles of general type **4.1** could be determined.

## *4.2. Synthesis of starting materials*

The key unlabelled and deuterium-labelled *N*-(*N*-phthalyl-*L*-leucinyl)pyrrole-2-carboxaldehydes **4.13a** and **4.13b** were prepared in yields of 87% and 91%, respectively, from the corresponding formylpyrroles **2.22a** and **2.22b** by acylation with *N*-phthalyl-*L*-leucine acid fluoride according to the method described by Šavrda *et al.* (Scheme 4.4).[3] The unlabelled *N*-(*N*-phthalyl-*L*-leucinyl)pyrrole-2-carboxaldehyde **4.13a** has previously been prepared, in a yield of 94%, by treating the formylpyrrole **2.22a** with *N*-phthalyl-*L*-leucine acid chloride and the acylation catalyst DMAP.[4] In our synthesis, however, the *N*-phthalyl-*L*-leucine acid fluoride was used in preference to the acid chloride to effect




**Scheme 4.4.** *Reagents and conditions*: (i) NaH, THF then *N*-Pht-*L*-Leu-F (**4.13a**, 87%), (**4.13b**, 91%); (ii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (**4.8a**, 88%); (iii) *S*-Alpine borane®, THF, rt (**4.8b**, 88%); (iii) *R*-Alpine borane®, THF, rt (**4.8c**, 90%).

*N*-acylation, since it is known that amino acid fluorides can be prepared, and then reacted, without any appreciable racemisation.[3,5] This was an important consideration in the subsequent configurational analysis (see later for a discussion). The unlabelled hydroxymethylpyrrole **4.8a** was then readily prepared, in a yield of 88%, by reducing the formyl group of **4.13a** with zinc borohydride. The corresponding deuterium-labelled analogues, **4.8b** and **4.8c**, were prepared, in yields of 88% and 90%, by reducing the deuterated formylpyrrole **4.13b** with either *S*-Alpine borane®, or *R*-Alpine borane®, respectively (Scheme 4.4).

The configurational purities at the deuterated methylene centres of **4.8b** and **4.8c** were determined to be >9:1 by integration of the pyrrole-CHD singlet resonances at δ 4.21 and 4.24, respectively (see first column, Table 4.1). This degree of configurational purity obtained at the deuterated methylene centres of **4.8b** and **4.8c** from the above stereoselective Alpine borane® reductions of the formylpyrrole **4.13b**, was consistent with the configurational purities obtained for previous examples (*ie* ~9:1 to >19:1) that were also prepared by Alpine borane® reductions (see Chapters 2 and 3 for details). Furthermore, since it is known that reduction of a deuterated aldehyde by *S*-Alpine borane® yields the *R*-alcohol, and that *R*-Alpine borane® reduction yields the *S*-alcohol,[6] the configurations at the deuterated methylene centres of **4.8b** and **4.8c** were able to be assigned as *R* and *S*, respectively (see Chapters 2 and 3 for further discussion). As a result, the observed *exo*-methylene resonances for **4.8b** and **4.8c** were able to be assigned as downfield for the *R*-isomer (*eg* **4.8b**), and upfield for the *S*-isomer (*eg* **4.8c**) (see first column, Table 4.1).

A potential complication in the above configurational analysis of the hydroxymethylpyrroles **4.8b** and **4.8c** arises due to the possibility of epimerisation at the leucine α-centre during the synthetic sequence. Any such epimerisation would lead to a decrease in the observed configurational purity at the deuterated methylene centres of **4.8b** and **4.8c** (see later for a discussion). However, the observed configurational purities of >9:1 for **4.8b** and **4.8c** would suggest that negligible epimerisation had occurred at this

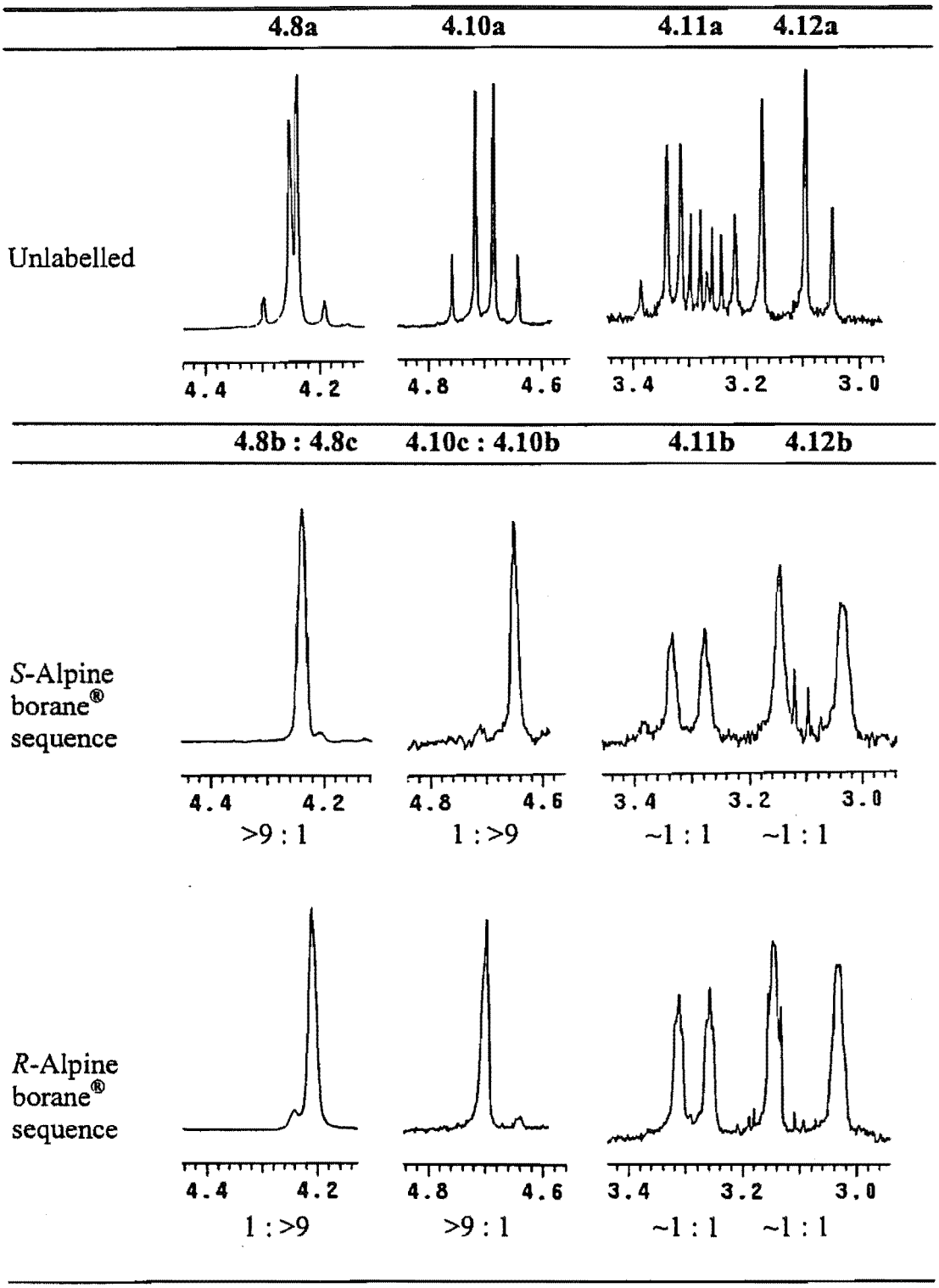



**Table 4.1.** $^1$H NMR resonances [$CD_3CN$, $CHCl_3$ internal standard (δ 7.25)] for the pyrrole *exo*-methylene group of **4.8** and the hydrolysis products **4.10**, **4.11** and **4.12** [KOH and *S*-(+)-*sec*-butylamine added]. In the spectra of **4.8a-c**, coupling of the signals with the OH proton were removed by homonuclear decoupling.

centre, presumably due to the use of *N*-phthalyl-*L*-leucine acid fluoride in the crucial *N*-acylation step (see Scheme 4.4). A subsequent attempt was made to analyse the configurational purity at the leucine α-centre of the hydroxymethylpyrroles **4.8** by preparing the *O*-camphanate derivatives of these compounds, in an analogous procedure to that discussed in Chapter 2.3. However, the conditions of the esterification reaction resulted in epimerisation at the leucine α-centre, thus complicating the analysis (see later for a discussion).

### *4.3. Hydrolysis study of* N-*(*N*-phthalyl*-L-*leucinyl)hydroxymethylpyrroles 4.8*

The mechanism of hydrolysis of compounds **4.8** was then studied by $^1$H NMR spectroscopy.[2] In a typical experiment, an equivalent of potassium hydroxide in $D_2O$ (approx 10 μL) was added to a solution of either **4.8a**, **4.8b** or **4.8c** (generally 3 mg) in $CD_3CN$ (150 μL) containing $CHCl_3$ as an internal standard (δ 7.25), and an equivalent of *S*-(+)-*sec*-butylamine (external nucleophile). The ratio of the *N*-acyl **4.8**, *O*-acyl **4.10** and aminomethylpyrroles **4.11** and **4.12** was then monitored by $^1$H NMR spectroscopy over time (*ca.* 18 hours). The *exo*-methylene proton resonances of these compounds are shown in Table 4.1. The $^1$H NMR spectrum taken immediately after the addition of the potassium hydroxide showed a mixture of the starting material **4.8**, *O*-acyl intermediate **4.10** and aminomethylpyrrole end products **4.11/4.12** in a typical ratio of 1 : 1 : 1. $^1$H NMR spectra of the mixtures after 18 hours revealed that **4.8** and **4.10** had been completely converted to the pyrrole amines **4.11** and **4.12**. The identity of the aminomethylpyrroles **4.11** and **4.12** as the end products of this hydrolysis reaction was subsequently confirmed by an independent synthesis of these compounds (see Section 4.4). This independent synthesis also allowed the assignment of the aminomethylpyrrole *exo*-methylene proton resonances as upfield for **4.12** relative to **4.11** (see third column, Table 4.1).

The key points to note from the results shown in Table 4.1 are that the configurations and stereochemical integrity (>9:1) of the starting materials **4.8b** and **4.8c** are retained in the corresponding *O*-acyl intermediates **4.10b** and **4.10c** (rows 2 and 3, Table 4.1). As discussed above, the formation of the *O*-acylpyrrole **4.10** could occur by either an intramolecular acyl transfer via the tetrahedral intermediate **4.9** [Scheme 4.3, pathway (*a*), retention of configuration], by the trapping of the azafulvene **4.3** with released *N*-phthalyl-*L*-leucine [Scheme 4.3, pathway (*b*), epimerisation at the *exo*-methylene], or by an $S_N2$ like displacement [Scheme 4.3, pathway (*c*), inversion of configuration]. The fact that the configurational purities at the deuterated methylene centres of **4.10b** and **4.10c** are intact, and also did not change with time, implies that these species are not in equilibrium with the azafulvene **4.3**, hence reaction via pathway (*b*) is not operating. Secondly, an $S_N2$ displacement at the *exo*-methylene position of a hydroxymethylpyrrole has only been observed in extreme examples using a combination of Mitsunobu reaction conditions and an *N*-triflyl substituent to strongly deactivate the pyrrole ring, and hence suppress azafulvene formation (see Chapter 2.4 for a discussion). In contrast, we have found that an *N*-acyl group was not sufficiently deactivating to promote such an $S_N2$ displacement, and hence a reaction of the type shown in pathway (*c*) of Scheme 4.3 is chemically unlikely under the conditions of the hydrolysis experiment. Pathway (*a*) in Scheme 4.3, however, is consistent with both our previous work, and the observed results, *ie* the formation of **4.10** occurs with retention of configuration at the deuterated methylene centre. A second point to note is that equal mixtures of the *R*- and *S*-deuterium-labelled pyrrole amines **4.11b** and **4.12b** were produced as the end products in the hydrolysis reactions of **4.8b** and **4.8c**, a result clearly consistent with the intermediacy of the azafulvene **4.3** [Scheme 4.3, pathway (*d*)]. Finally, as expected, the two oppositely labelled series **4.8b** and **4.8c** gave complementary results (rows 2 and 3, Table 4.1).

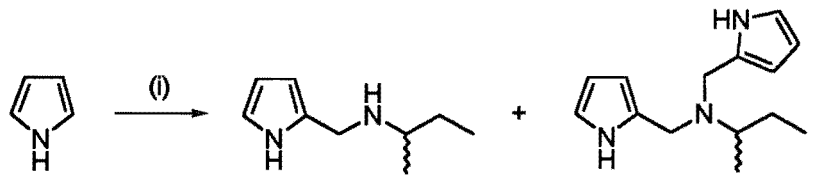
Another key point to note from the results shown in Table 4.1 is that in the case of the *O*-acylpyrroles **4.10b** and **4.10c**, the methylene resonance is upfield for the *R*-isomer

(*eg* **4.10b**) relative to the *S*-isomer (*eg* **4.10c**). This is the reverse of what was observed for the *N*-acylpyrroles **4.8b** and **4.8c**. A similar reversal in the observed relative positions of the *R*- and *S*-methylene resonances was noted during the *O*-mesylation and chlorination reactions of the *R*- and *S*-[*methylene-d*$_1$]-*N*-camphorsulfonyl hydroxymethylpyrroles **3.45b** and **3.45c** (see Chapter 3.4).

In summary, the above observations strongly suggest that the *O*-acylpyrrole **4.10** is most likely formed via an intramolecular acyl transfer on hydrolysis of the *N*-(*N*-phthalyl-*L*-leucinyl)hydroxymethylpyrroles **4.8**. Evidence for the subsequent release of an azafulvene **4.3** is gained from the observed scrambling of the deuterium-label at the *exo*-methylene positions of **4.11** and **4.12** on trapping with an external nucleophile – either *S*-(+)-*sec*-butylamine or **4.11**, respectively.

## *4.4. Independent synthesis of aminomethylpyrroles 4.11 and 4.12*

To confirm the identity of the aminomethylpyrroles **4.11** and **4.12** as the end products in the above hydrolysis study, and to allow the correct assignment for the respective *exo*-methylene resonances in the $^1$H NMR spectra of the hydrolysis experiments, the pyrrole amines **4.11** and **4.12** were independently synthesised. The aminomethylpyrrole **4.11** was prepared by the method described by Herz *et al.*[7] In this method, pyrrole was treated with a slight excess (1.05 equivalents) of a solution of (±)-*sec*-butylamine hydrochloride in 37% aqueous formalin to give the aminomethylpyrrole **4.11** in a yield of 62% (Scheme 4.5). Next, the aminomethylpyrrole **4.12** was prepared by an analogous procedure to that described above, in which a two molar excess of both pyrrole and formalin were used in order to enable the further substitution of the initially prepared amine **4.11**. In this way, the disubstituted aminomethylpyrrole **4.12** was prepared in a yield of 20%, along with the monosubstituted product **4.11** in 76% yield (Scheme 4.5).




**4.11** **4.12**

**Scheme 4.5.** *Reagents and conditions*: (i) (±)-*sec*-$BuNH_2$.HCl, 37% aq. formalin (**4.11**, 62%) or (**4.11**, 76%; **4.12**, 20%).

The $^1H$ NMR spectra of these reference aminomethylpyrroles **4.11** and **4.12** revealed that, in both cases, the pyrrole *exo*-methylene group was observed as an AB quartet, resonating at δ 3.73 and 3.81, and 3.49 and 3.65, respectively. As a result, this enabled the assignment of the aminomethylpyrrole *exo*-methylene resonances in the $^1H$ NMR spectra of the hydrolysis experiments as upfield for **4.12** relative to **4.11** (see third column, Table 4.1).

## *4.5. Attempted configurational analysis of the leucine α-centre of* N-(N-phthalylleucinyl)hydroxymethylpyrroles

As discussed previously, a possible complication in the configurational analysis at the deuterated methylene centre of the hydroxymethylpyrroles **4.8b** and **4.8c** arises due to the possibility of epimerisation at the leucine α-centre during the synthetic sequence. The consequences of any such epimerisation at the leucine α-centre requires an examination of all the possible isomers which could arise during the synthesis of compounds **4.8b** and **4.8c** (see Figure 4.1).

If the preparation of the formylpyrrole **4.13b** and its subsequent reduction to the deuterated hydroxymethylpyrroles **4.8** occurred without epimerisation at the leucine α-centre, then the only isomers that would be produced would be the *L*-leucine isomers **4.8b** and **4.8c** (see Scheme 4.4 and Figure 4.1). However, if epimerisation had occurred

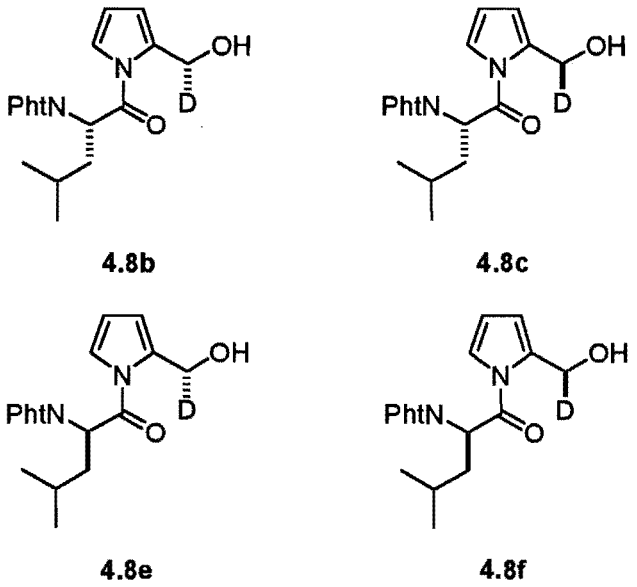
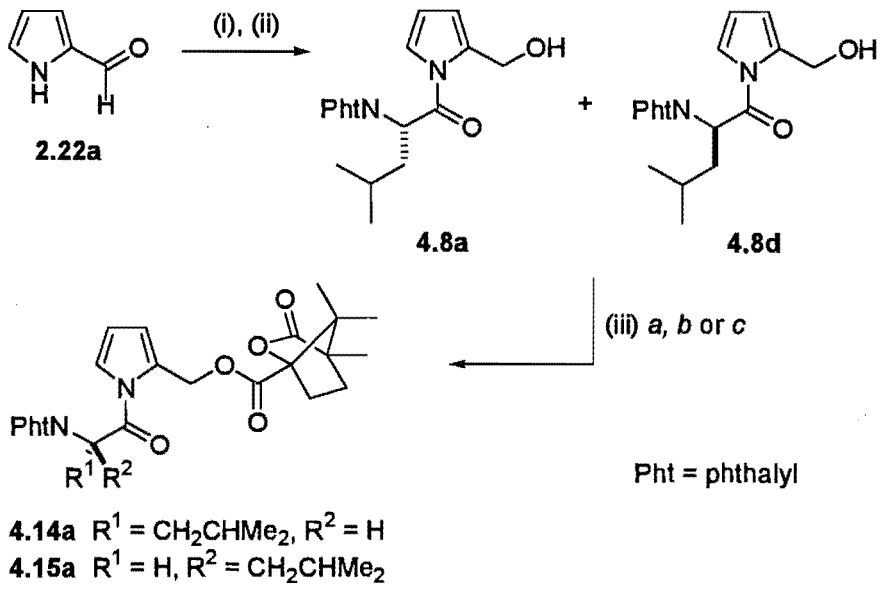


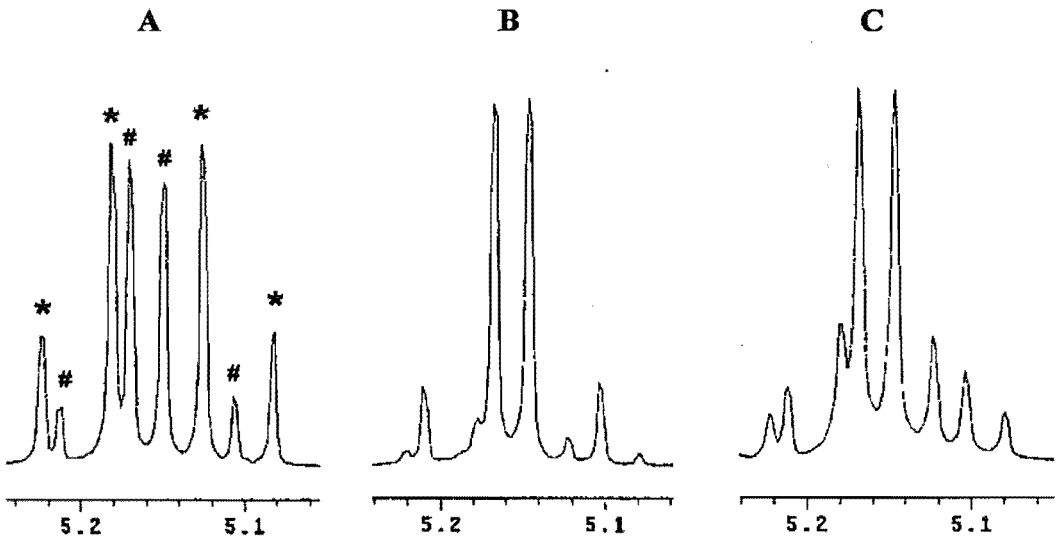

**Figure 4.1.** Isomers of [*methylene-d*$_1$]-*N*-(*N*-phthalylleucinyl)hydroxymethylpyrroles.

at the leucine α-centre, then the *D*-leucine isomers **4.8e** and **4.8f** would also be produced. If in this latter case the reduction had been carried out using *R*-Alpine borane®, which is known to yield alcohols with the *S*-configuration,[6] the major products formed would be the isomers **4.8c** and **4.8f**, which have the *S*-configuration at the deuterated methylene centre. In addition, a small amount of the *R*-isomers **4.8b** and **4.8e** would also be expected, since the reduction is not completely stereospecific. An important point to note, however, is that the isomers **4.8c/4.8e** and **4.8b/4.8f** are enantiomeric pairs, and as such, are indistinguishable by $^1$H NMR spectroscopy. As a result, it would be impossible to differentiate the contribution that each isomer of the enantiomeric pair makes to the particular methylene resonance. In the above example, the greater the degree of epimerisation at the leucine α-centre, the greater the contribution the **4.8f** isomer would make to the downfield methylene resonance. In the absence of any such epimerisation at leucine, this resonance is attributed solely to the *R*-isomer **4.8b**, and therefore gives a direct measure of the chiral reduction efficiency. However, with an increase in the downfield resonance due to **4.8f**, the observed epimeric purity at the deuterated methylene centre of **4.8** would be decreased, and as a result, the apparent efficiency of the chiral reduction would also appear diminished.

An attempt was subsequently made to determine whether any such epimerisation had occurred at the leucine α-centre during the preparation of **4.8**. This was attempted by the introduction of a further chiral handle, namely the previously described (1*S*,4*R*)-(–)-camphanate group, onto the hydroxymethyl position of **4.8**, in an analogous procedure to that discussed in Chapter 2.3. In this way, the *exo*-methylene resonances resulting from both the *L*- and *D*-leucine isomers (*ie* **4.14a** and **4.15a**, Scheme 4.6) could be discriminated, which would therefore allow the configurational purity at the leucine α-centre of **4.8** to be established. To enable this analysis, a 1:1 mixture of the *L*- **4.8a** and *D*- **4.8d** *N*-(*N*-phthalylleucinyl)hydroxymethylpyrroles was prepared from pyrrole-2-carboxaldehyde **2.22a** in 81% yield (2 steps) by N-substitution with racemic *N*-phthalyl-*DL*-leucine acid fluoride, followed by zinc borohydride reduction of the formyl group (Scheme 4.6). Subsequent esterification of this 1:1 mixture of **4.8a** and **4.8d** with (1*S*,4*R*)-(–)-camphanoyl chloride using standard procedures then gave an equal mixture of the camphanates **4.14a** and **4.15a** in a combined yield of 83%. $^1$H NMR spectral




**Scheme 4.6.** *Reagents and conditions*: (i) NaH, THF then *N*-Pht-*DL*-Leu-F; (ii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (81% for 2 steps); (iii) DMAP, Hünig's base (1*S*,4*R*)-(–)-camphanoyl chloride and either (*a*) 1:1 **4.8a/4.8d**, 24 h (83%), (*b*) **4.8a**, 24 h (94%), or (*c*) **4.8a**, 48 h (79%).




4.14a/4.15a
1 : 1

4.14a/4.15a
~9 : 1

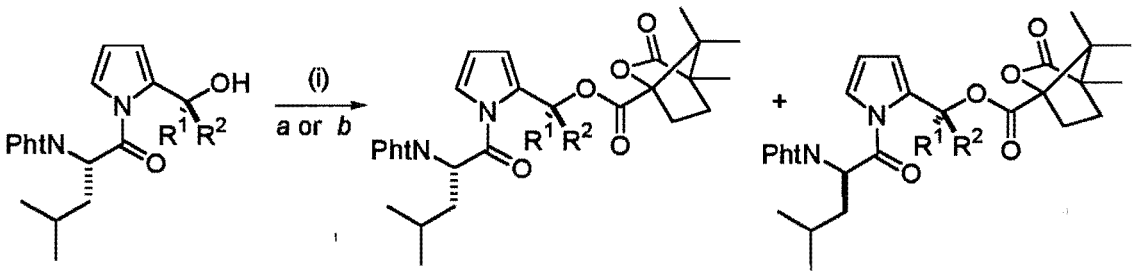
4.14a/4.15a
~5 : 1

**Figure 4.2.** $^1$H NMR resonances ($CD_3CN$) for the pyrrole *exo*-methylene group of the camphanates **4.14a** (shown in Spectrum A by #) and **4.15a** (shown in Spectrum A by ★).

analysis of this reference camphanate sample revealed that, in both the *L*- and *D*-leucine isomers, the pyrrole *exo*-methylene group was observed as an AB quartet, resonating at δ 5.10 and 5.20 for the *D*-isomer (*ie* **4.15a**), and at δ 5.13 and 5.19 for the *L*-isomer (*ie* **4.14a**) (see Figure 4.2, Spectrum A). Next in the key experiment, the unlabelled hydroxymethylpyrrole **4.8a**, prepared as detailed in Scheme 4.4, was esterified with (1*S*,4*R*)-(–)-camphanoyl chloride by the same method as used above, to give a mixture of the camphanates **4.14a** and **4.15a** in a combined yield of 94%. The ratio of **4.14a** and **4.15a** observed by $^1$H NMR spectral analysis of this camphanate sample was ~9:1 (Figure 4.2, Spectrum B), indicating a small amount of epimerisation had occurred at the leucine α-centre. However, it was uncertain whether this epimerisation at the leucine α-centre had occurred during the preparation of **4.8a**, or was in fact a consequence of this camphanate analysis. This second possibility was consistent with the work of Šavrda *et al.*,[3] where it was found that during a peptide coupling reaction, a triethylamine favoured α-H exchange led to a substantial amount of epimerisation in an amino acid which

possessed several electron withdrawing groups. Consequently, the epimerisation we observed from our camphanate analysis could in fact be due to an analogous Hünig's base favoured α-H exchange during the camphanate preparation. In order to establish whether or not this was the case, the esterification of **4.8a** was repeated as before, but with the reaction time increased from 24 to 48 hours, so as to enable the possibility of further epimerisation to occur. Esterification under these conditions gave a mixture of the camphanates **4.14a** and **4.15a** in a combined yield of 79%. The ratio of **4.14a** and **4.15a** observed by $^1$H NMR spectral analysis of the resulting camphanate sample was ~5:1 (Figure 4.2, Spectrum C). This decrease in the ratio of **4.14a** relative to **4.15a** with an increase in reaction time therefore suggests that the observed epimerisation is in fact a consequence of the attempted camphanate analysis. As a result, this method cannot be used to give an accurate measure of the configurational purity at the leucine α-centre of the starting hydroxymethylpyrrole **4.8a**.

In a final experiment, the above camphanate analysis was repeated using separate samples of the deuterium-labelled hydroxymethylpyrroles **4.8b** and **4.8c**, prepared as detailed in Scheme 4.4, in order to discriminate by $^1$H NMR spectroscopy all of the resulting isomers formed (*ie* **4.14b-c** and **4.15b-c**). Consequently, separate samples of the deuterated hydroxymethylpyrroles **4.8b** and **4.8c**, which had known configurational




**4.8b** $R^1$ = D, $R^2$ = H
**4.8c** $R^1$ = H, $R^2$ = D

**4.14b** $R^1$ = D, $R^2$ = H
**4.14c** $R^1$ = H, $R^2$ = D

**4.15b** $R^1$ = D, $R^2$ = H
**4.15c** $R^1$ = H, $R^2$ = D

**Scheme 4.7.** *Reagents and conditions*: (i) DMAP, Hünig's base (1*S*,4*R*)-(–)-camphanoyl chloride, 24 h and either (*a*) **4.8b** (74%) or (*b*) **4.8c** (86%).

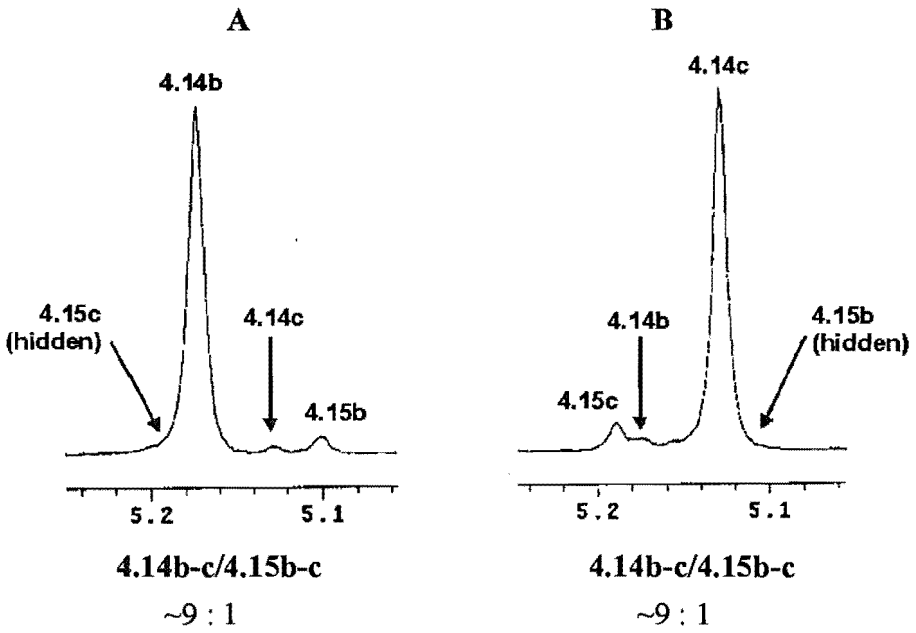



**Figure 4.3.** $^1$H NMR resonances ($CD_3CN$) for the pyrrole *exo*-methylene group of the deuterated camphanates **4.14b-c** and **4.15b-c** derived from **4.8b** (Spectrum A) and **4.8c** (Spectrum B).

purities of >9:1 (see earlier for a discussion), were esterified with (1*S*,4*R*)-(–)-camphanoyl chloride under standard conditions to give mixtures of the corresponding labelled camphanates **4.14b-c** and **4.15b-c** in combined yields of 74% and 86%, respectively (Scheme 4.7).

The methylene resonances for the deuterium-labelled camphanates **4.14b-c** and **4.15b-c** were observed in the $^1$H NMR spectra as singlets with distinguishable chemical shifts at δ 5.17 and 5.13, and 5.10 and 5.19, respectively (Figure 4.3). These resonances were assigned by a comparison with the $^1$H NMR spectral data obtained previously for the unlabelled *L*- and *D*-leucine camphanates **4.14a** and **4.15a**, respectively (see Figure 4.2). Furthermore, the ratio of the deuterated camphanates **4.14b-c** and **4.15b-c** derived from **4.8b** (Figure 4.3, Spectrum A) and **4.8c** (Figure 4.3, Spectrum B) were determined to be ~9:1 by $^1$H NMR spectral analysis. This was in line with the isomer ratio of ~9:1 we had observed previously in the unlabelled camphanates **4.14a** and **4.15a** (see Figure

4.2, Spectrum B). This again strongly suggests that the observed epimerisation at the leucine α-centre of **4.8b** and **4.8c** was in fact a consequence of the attempted camphanate analysis. An interesting point to note from the spectra shown in Figure 4.3 is that in the case of the *L*-leucine camphanate **4.14**, the *exo*-methylene resonance is upfield for the *S*-isomer (*eg* **4.14c**) relative to the *R*-isomer (*eg* **4.14b**). However, in the case of the *D*-leucine camphanate **4.15**, these configurations are reversed, with the *exo*-methylene resonance upfield for the *R*-isomer (*eg* **4.15b**) relative to the *S*-isomer (*eg* **4.15c**).

## *4.6. Conclusion*

We have determined the stereochemical fate of the deuterium-label in **4.8b** and **4.8c** upon the potassium hydroxide catalysed hydrolysis of these compounds in the presence of an external nucleophile [*S*-(+)-*sec*-butylamine]. The observation that both the stereochemistry and configurational purity of the deuterated methylene centres of **4.8b** and **4.8c** did not change upon formation of the *O*-acyl intermediates **4.10b** and **4.10c**, respectively, strongly suggests that the *O*-acylpyrrole **4.10** is formed by an intramolecular *N*- to *O*-acyl transfer, via the tetrahedral intermediate **4.9**, with retention of configuration. Evidence for the subsequent release of an azafulvene **4.3** was gained from the observed scrambling of the deuterium-label at the *exo*-methylene positions of **4.11** and **4.12** on trapping with an external nucleophile – either *S*-(+)-*sec*-butylamine or **4.11**, respectively. The identity and assignments of the aminomethylpyrroles **4.11** and **4.12** were subsequently confirmed by an independent synthesis of these compounds. Finally, an attempt to analyse the configurational purity at the leucine α-centre of **4.8**, by the preparation of the *O*-camphanates, resulted in epimerisation at the leucine α-centre.

## 4.7. References for chapter four


1 Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*. CRC Press: Florida, 1988, Vol 1, pp95-156.

2 Abell, A. D.; Litten, J. C. *Tetrahedron Lett.* **1992**, *33*, 3005.

3 Šavrda, J.; Chertanova, L.; Wakselman, M. *Tetrahedron* **1994**, *50*, 5309.

4 Abell, A. D.; Litten, J. C. *Aust. J. Chem.* **1993**, *46*, 1473.

5 Bertho, J. –N.; Loffet, A.; Pinel, C.; Reuther, F.; Sennyey, G. *Tetrahedron Lett.* **1991**, *32*, 1303.

6 Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352.

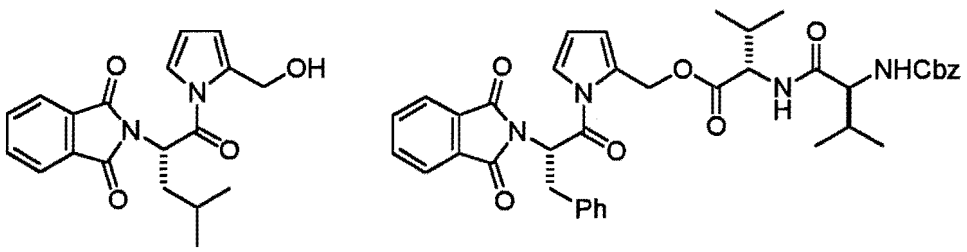
7 Herz, W.; Dittmer, K.; Cristol, S. J. *J. Am. Chem. Soc.* **1947**, *69*, 1698.

# CHAPTER FIVE

# DEVELOPMENT OF HYDROXYMETHYLPYRROLES AS SERINE PROTEASE INHIBITORS

## 5.1. Introduction

Serine proteases are a subclass of proteolytic enzymes which are characterised by the presence of a catalytic serine amino acid residue in the enzyme active site. The natural function of such enzymes is to catalyse the hydrolytic cleavage of peptide bonds in proteins and polypeptides (see Chapter 1.4 for further discussion and for the mechanism of serine protease catalysed hydrolysis of a peptide bond). An imbalance in the activity of serine proteases has been implicated in the initiation and progression of many human diseases, such as emphysema, adult respiratory distress syndrome (ARDS), pancreatitis, rheumatoid arthritis, inflammation and digestive disorders.[1] Consequently, much research has been directed towards the development of selective inhibitors of serine proteases, as such compounds could then provide a means by which these, and related conditions, could be treated. In light of this, the *N*-acylhydroxymethylpyrroles **4.8a** and **5.1** have previously been developed from work in our laboratories as amino acid analogue, mechanism-based inhibitors of serine proteases (Figure 5.1).[2]




**4.8a** **5.1** Cbz = carbobenzyloxy

**Figure 5.1.** Previously reported hydroxymethylpyrrole-based inhibitors of serine proteases.

A mechanism-based inhibitor is a chemically non-reactive or 'latent' compound which is sufficiently similar in structure to the natural substrate of a target enzyme such that it is able to be bound to the enzyme's active site. Once in the active site it can be

acted upon by the enzyme's normal catalytic pathway to produce a reactive species, which can then result in the specific and efficient inhibition of the target enzyme. In the case of *N*-acylhydroxymethylpyrrole-based inhibitors described above, the enzymatic removal of the *N*-acyl group leads to the formation of a highly reactive azafulvene in the active site. This azafulvene is therefore the chemical species responsible for the inherent (or latent) reactivity of these compounds (see Chapter 1.4 for the proposed mechanism of action of this class of mechanism-based inhibitor).

The *N*-acylhydroxymethylpyrroles **4.8a** and **5.1**, however, lack the extended peptide-like sequences usually found in the natural substrates of α-chymotrypsin and other serine proteases. This would result in these peptidomimetics having a lowered binding affinity for the enzyme active site due to reduced enzyme-substrate recognition. As a consequence, the *N*-acylhydroxymethylpyrroles **4.8a** and **5.1** would most likely have a lowered, or non-selective, inhibitory effect on serine proteases. In the case of α-chymotrypsin, a large well defined hydrophobic pocket at the enzyme's primary specificity pocket ($S_1$) is known to accept and readily cleave substrates with aromatic residues, such as phenylalanine, tyrosine and tryptophan, positioned at the primary amino acid binding site ($P_1$) (see Figure 5.2 and Chapter 1.4 for a definition of this S/P nomenclature). α-Chymotrypsin is also known to accept and cleave, albeit less readily, substrates with hydrophobic aliphatic residues positioned at the $P_1$ site. Thus, cleavage can occur on the carboxyl side of isoleucine, leucine and valine, although these represent much less favoured cleavage sites. However, enzyme-substrate recognition is not based solely on the $S_1$-$P_1$ interaction, but is in fact influenced by neighbouring and longer range secondary interactions (see Chapter 1.4 for further discussion). Due to this importance of both primary and secondary interactions in enzyme-substrate (or inhibitor) recognition, subsequent work, detailed in this chapter, was centred on developing new methodologies to incorporate the hydroxymethylpyrrole-moiety into an extended peptide-like sequence that was more closely based on the natural substrate (see Figure 5.2). By doing this, it was hoped that the newly developed inhibitor molecules would have increased enzyme

specificity and selectivity, due to an improvement in the recognition and subsequent binding of these inhibitor molecules in the active site of the target enzyme.

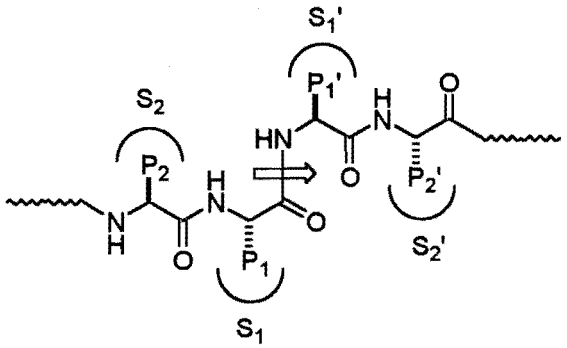



**Figure 5.2.** Natural substrate of a serine protease showing the enzyme-substrate recognition. The bond to be cleaved is shown by an arrow.

An increase in the enzyme recognition of the inhibitor molecule could conceivably be achieved by extending the hydroxymethylpyrrole-moiety at either the 2- or 5-positions (or in both directions), as well as by extending the *N*-acyl chain (see Figure 5.3). Work detailing the synthetic efforts undertaken in these areas is presented in the following sections of this chapter.

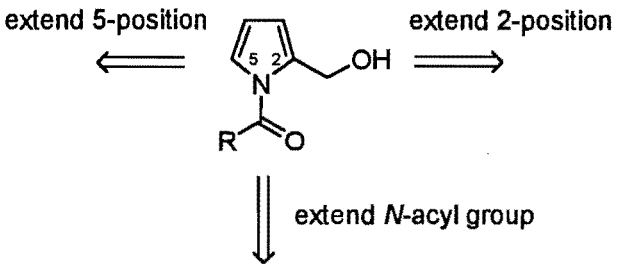


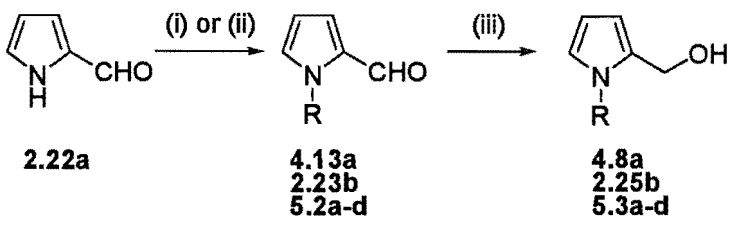

**Figure 5.3.** Potential ways to increase enzyme recognition of the inhibitor molecule.

## 5.2. *Determination of optimal pyrrole* N-*substituent and leaving group for enzyme inhibition*

All previous studies on hydroxymethylpyrroles as mechanism-based inhibitors of serine proteases had employed an acyl group on nitrogen to deactivate the pyrrole, and hence suppress azafulvene formation.[2,3] This deactivation is critical to the proposed mechanism of action of these compounds, since release of the reactive azafulvene before delivery to the enzyme would decrease the effectiveness of the inhibitor. However, we had shown in our earlier studies that an *N*-acyl group was not the optimal substituent for suppressing this azafulvene formation. Instead, we had shown that *N*-sulfonyl groups, such as triflyl or mesyl, were more effective in deactivating the pyrrole ring than either *N*-alkoxycarbonyl or *N*-acyl groups, with the *N*-triflyl group having the best deactivating, and hence azafulvene suppressing, ability (see Chapter 2.4 for a discussion). In light of this, we decided to investigate whether *N*-sulfonyl substituted hydroxymethylpyrroles would show improved, or indeed if any, inhibition activity against serine proteases. The underlying issue in such a substitution, however, is the balance between chemical stability and useful chemical reactivity. Thus, while the *N*-sulfonyl substituted hydroxymethylpyrrole may indeed be more stable than the corresponding *N*-acyl substituted hydroxymethylpyrrole, it may in fact be too stable to undergo the crucial enzyme catalysed hydrolytic cleavage of the *N*-substituent, thereby preventing the release of the latent reactivity of the inhibitor molecule (*ie* azafulvene formation). To resolve this issue we decided to prepare a range of simple *N*-acyl and *N*-sulfonyl substituted hydroxymethylpyrroles and then assay these compounds for potential inhibition activity against the serine protease, α-chymotrypsin. In this way, we hoped to determine the respective abilities of *N*-acyl and *N*-sulfonyl substituted hydroxymethylpyrroles to act as mechanism-based inhibitors of serine proteases. The compounds that were selected for this preliminary investigation were the *N*-acylhydroxymethylpyrroles **4.8a** and **5.3a**, and

the *N*-sulfonylhydroxymethylpyrroles **2.25b** and **5.3b-d** (see Scheme 5.1). These compounds, which varied in both size and degree of unsaturation of the *N*-substituent, were selected for analysis since the target enzyme in the subsequent assay, α-chymotrypsin, is known to cleave preferentially at aromatic and hydrophobic residues. In this way, it was hoped that the optimal *N*-substituent for binding in the enzyme's primary specificity pocket ($S_1$) could be determined. In addition, the *N*-(*N*-phthalyl-*L*-leucinyl)hydroxymethylpyrrole **4.8a** was selected for analysis as it had previously been prepared and assayed in an earlier study.[3] As a consequence, this compound provided a reference sample by which to compare the inhibition results from this investigation with those of the earlier study.

The starting *N*-substituted formylpyrroles **4.13a**, **2.23b** and **5.2b-d** were prepared, in yields of 87%, 93%, 97%, 56% and 84%, respectively, by treating the formylpyrrole **2.22a** with sodium hydride, and then reacting the generated pyrrolyl anion with either *N*-phthalyl-*L*-leucine acid fluoride, methanesulfonyl chloride, phenylsulfonyl chloride, β-*trans*-styrenesulfonyl chloride or 2-phenylethanesulfonyl chloride (Scheme 5.1). The remaining *N*-hydrocinnamoyl formylpyrrole **5.2a** was prepared in 79% yield by treating the formylpyrrole **2.22a** with hydrocinnamoyl chloride in the presence of the acylation catalyst 4-dimethylaminopyridine (DMAP). The *N*-substituted hydroxymethylpyrroles

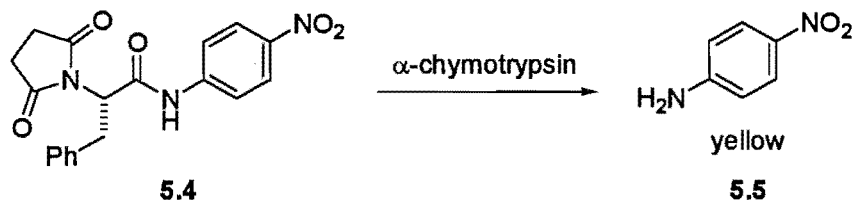



| | R |
|---|---|
| **4.13a/4.8a** | *N*-Pht-*L*-Leu |
| **2.23b/2.25b** | $SO_2Me$ |
| **5.2a/5.3a** | $CO(CH_2)_2Ph$ |
| **5.2b/5.3b** | $SO_2Ph$ |
| **5.2c/5.3c** | $SO_2CH{:}CHPh$ |
| **5.2d/5.3d** | $SO_2(CH_2)_2Ph$ |

**Scheme 5.1.** *Reagents and conditions:* (i) NaH then either *N*-Pht-*L*-Leu-F (**4.13a**, 87%) or $MeSO_2Cl$ (**2.23b**, 93%) or $PhSO_2Cl$ (**5.2b**, 97%) or $PhCH{:}CHSO_2Cl$ (**5.2c**, 56%) or $Ph(CH_2)_2SO_2Cl$ (**5.2d**, 84%); (ii) DMAP, Hünig's base, $CH_2Cl_2$ then $Ph(CH_2)_2COCl$ (**5.2a**, 79%); (iii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (**4.8a**, 88%), (**2.25b**, 91%), (**5.3a**, 93%), (**5.3b**, 92%), (**5.3c**, 95%), (**5.3d**, 94%).

**4.8a**, **2.25b** and **5.3a-d** were then readily prepared, in yields of 88%, 91%, 93%, 92%, 95% and 94%, respectively, by reducing the corresponding formylpyrroles **4.13a**, **2.23b** and **5.3a-d** with zinc borohydride.

The hydroxymethylpyrroles **4.8a**, **2.25b** and **5.3a-d** were tested as potential inhibitors of α-chymotrypsin by the method outlined by Cannell *et al.*[4] This simple α-chymotrypsin assay was based on a colorimetric technique, where enzymatic release of yellow-coloured 4-nitroaniline **5.5** from the colourless *N*-succinyl-*L*-phenylalanine-4-nitroanilide **5.4** substrate, indicates activity (Scheme 5.2). The advantages of this assay procedure are that it is rapid, and it also allows for a large number of samples to be tested simultaneously by using a 96 well reaction plate. For full details of the assay procedure see Chapter 8.5.3.




**5.4** **5.5**

**Scheme 5.2.** Enzymic breakdown of the colorimetric substrate.

Solutions of the pyrroles **4.8a**, **2.25b** and **5.3a-d** were made to 1250 μg $mL^{-1}$, 125 μg $mL^{-1}$ and 12.5 μg $mL^{-1}$ as standard solutions in methanol. The pyrrole test solution, distilled water, Tris-HCl buffer (pH 7.6) and a solution of α-chymotrypsin in Tris-HCl buffer (pH 7.6) were added to each well of a microtitre plate, and the resulting solutions were pre-incubated at 37 °C for 30 min. The substrate **5.4** was then added, and the absorbance was read at 405 nm at t = 0, and after incubation at 37 °C when significant colour change had taken place. Control samples were included in all assay cases. Each sample was tested in triplicate and average absorbances were used to calculate the percentage inhibitions. The results obtained in this α-chymotrypsin assay are presented

in Table 5.1. The previously determined inhibition results for compound **4.8a** are given in parentheses directly after the current results for **4.8a**.

| Compound | % Inhibition | | |
|---|---|---|---|
| | 1250 μg $mL^{-1}$ | 125 μg $mL^{-1}$ | 12.5 μg $mL^{-1}$ |
| **4.8a** | 40 | 10 (25) | 0 (20) |
| **5.3a** | 25 | 10 | 10 |
| **2.25b** | 0 | 0 | 0 |
| **5.3b** | 35 | 20 | 5 |
| **5.3c** | 30 | 15 | 5 |
| **5.3d** | 20 | 10 | 5 |

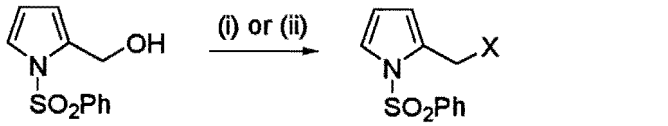
**Table 5.1.** Results of α-chymotrypsin assay (results rounded to nearest 5%)

The results detailed in Table 5.1 indicate that the *N*-acyl and *N*-sulfonyl hydroxymethylpyrrole derivatives **4.8a** and **5.3a-d** were modest inhibitors of α-chymotrypsin. In the present study, the degree of inhibition of the test enzyme for compound **4.8a** was 10% and 0% at the 125 μg $mL^{-1}$ and 12.5 μg $mL^{-1}$ concentrations, respectively. In contrast, the previously determined inhibition results for compound **4.8a** were 25% and 20%, respectively, at the same concentrations.[3] This difference in the inhibitory activity of **4.8a** in the two studies was attributed to the inherent differences between the two assay procedures, such as the use of different samples of enzyme and substrate, and to variations in the pH of the respective assay test solutions. Regardless of this difference, however, the important point to note from the results of this present analysis was that the *N*-arylsulfonyl substituted hydroxymethylpyrroles **5.3b-d** all showed activity against α-chymotrypsin. Moreover, the inhibitory activities of these *N*-sulfonyl derivatives were approximately on a par with those of the *N*-acyl derivatives **4.8a** and **5.3a**, at all the concentrations tested. This indicates that *N*-sulfonyl hydroxymethylpyrroles, as with the analogous *N*-acyl derivatives, are indeed able to promote the inhibition of α-chymotrypsin, and also possibly of other serine proteases.

The one exception to this, though, was the *N*-mesyl derivative **2.25b**, which showed no inhibition activity at any of the inhibitor concentrations tested. This was thought to be due to the mesyl group being a poor substituent for recognition by α-chymotrypsin, due to the lack of a suitable aromatic or hydrophobic group to fit in the enzyme's primary specificity pocket ($S_1$). Of the arylsulfonyl groups tested in this analysis, the phenylsulfonyl group (*ie* compound **5.3b**) was found to have a slightly higher inhibitory activity against α-chymotrypsin. However, the differences in the enzyme inhibition activities between the different hydroxymethylpyrroles **5.3b-d** were too small to allow a definitive assignment for the optimal *N*-arylsulfonyl group for enzyme inhibition.

Having established that *N*-arylsulfonyl hydroxymethylpyrroles were able to effectively inhibit α-chymotrypsin under these assay conditions, we next turned our attention to determining which leaving group at the 2-methyl position was optimal for this enzyme inhibition. Up to this time, the only pyrrole derivative which had been assayed with something other than a hydroxyl group at the 2-methyl position was the compound **5.1** (Figure 5.1), which had an *N*-carbobenzyloxy-*L*-valyl-*L*-valine dipeptide esterified onto the 2-hydroxymethyl group. The subsequent assay of this derivative by the method described above, showed a 45% inhibition of α-chymotrypsin at an inhibitor concentration of 12.5 μg $mL^{-1}$. However, at the higher concentration of 125 μg $mL^{-1}$, the compound was observed to precipitate out of solution during the assay, and so no reliable inhibition data was able to be obtained for compound **5.1** at this concentration.[3]

The compounds that were selected for this investigation into the optimal leaving group on pyrrole for enzyme inhibition were the *N*-phenylsulfonylpyrroles **5.6a** and **5.6b**, which were substituted at the 2-methyl position with either a chloro or an *N*-phthalyl-*L*-leucinyl group, respectively (see Scheme 5.3). Consequently, the chloromethylpyrrole **5.6a** was prepared in quantitative yield by treating the hydroxymethylpyrrole **5.3b** with methanesulfonyl chloride and Hünig's base, while the *O*-acylpyrrole **5.6b** was prepared in 90% yield by the esterification of **5.3b** with *N*-phthalyl-*L*-leucine acid fluoride using standard procedures (Scheme 5.3).




**5.3b**

**5.6a** X = Cl
**5.6b** X = *N*-Pht-*L*-LeuCO$_2$

**Scheme 5.3.** *Reagents and conditions*: (i) Hünigs base, $MeSO_2Cl$ then 10% aq. HCl (**5.6a**, quant.); (ii) DMAP, Hünig's base, *N*-Pht-*L*-Leu-F, $CH_2Cl_2$ (**5.6b**, 90%).

The 2-substituted pyrroles **5.6a** and **5.6b** were then assayed as potential inhibitors of α-chymotrypsin by the colorimetric method outlined above. The results of this α-chymotrypsin assay, along with the previously determined inhibition results for compound **5.3b**, are presented in Table 5.2.

| **Compound** | **% Inhibition** | | |
|---|---|---|---|
| | 1250 μg mL$^{-1}$ | 125 μg mL$^{-1}$ | 12.5 μg mL$^{-1}$ |
| **5.3b** | 35 | 20 | 5 |
| **5.6a** | 5 | 5 | 0 |
| **5.6b** | 0* | 0* | 0 |

* precipitation occurred in reaction wells during assay

**Table 5.2.** Results of α-chymotrypsin assay (results rounded to nearest 5%).

The results detailed in Table 5.2 clearly show that of the leaving groups examined in this investigation, the hydroxymethyl group (*ie* compound **5.3b**) was by far the optimum group for enzyme inhibition activity. This result might be in line with our previous chemical studies on the hydrolytic cleavage of an *N*-substituted pyrrole, which found the initial step in this process to be an intramolecular *N*- to *O*- transfer of the *N*-substituent, to give a species analogous to **5.6b**. This was then followed by the subsequent release of an azafulvene – the chemical species postulated to be responsible for the enzyme inhibition activity of this class of inhibitor (see Chapter 4 for further

discussion). In contrast, compounds **5.6a** and **5.6b** have no such hydroxyl group available to enable this initial intramolecular *N*- to *O*- transfer of the *N*-substituent, and so would be unable to undergo hydrolytic cleavage by such a mechanism. Nevertheless, the chloromethylpyrrole **5.6a** did show a small degree of enzyme inhibition activity (*ca.* 5%) at the higher inhibitor concentrations of 1250 μg $mL^{-1}$ and 125 μg $mL^{-1}$. At this time, however, the exact mechanism of action for this compound remains unknown. Finally, the observation that the higher concentrations of the *O*-acylpyrrole **5.6b** precipitated in the reaction wells during the assay indicated that either this compound was unstable, or insoluble, in the assay conditions. However, at the concentration of **5.6b** at which no precipitation was observed to occur (*ie* 12.5 μg $mL^{-1}$), no enzyme inhibition activity was observed. The conclusion from these results, therefore, is that a hydroxymethyl group is the optimal group on pyrrole to effect enzyme inhibition. However, further work is required in order to develop a better understanding of the mechanisms involved in enzyme inhibition for this class of mechanism-based inhibitor.

## 5.3. *Preparation of pyrrole-based peptidomimetics*

In a final attempt to prepare pyrrole-based inhibitors of serine proteases that had the potential for increased enzyme recognition, work was centred on incorporating the hydroxymethylpyrrole-moiety into an extended peptide-like sequence. Consequently, a number of key potential synthetic targets, **5.7** – **5.12** (Figure 5.4), were identified for further investigation. These compounds represent examples of extension in the 2- and 5-position of the general structure shown in Figure 5.3. The following sections of work detail the methodologies that were attempted in the preparation of derivatives of these compounds, and their subsequent testing as potential serine protease inhibitors (where applicable).

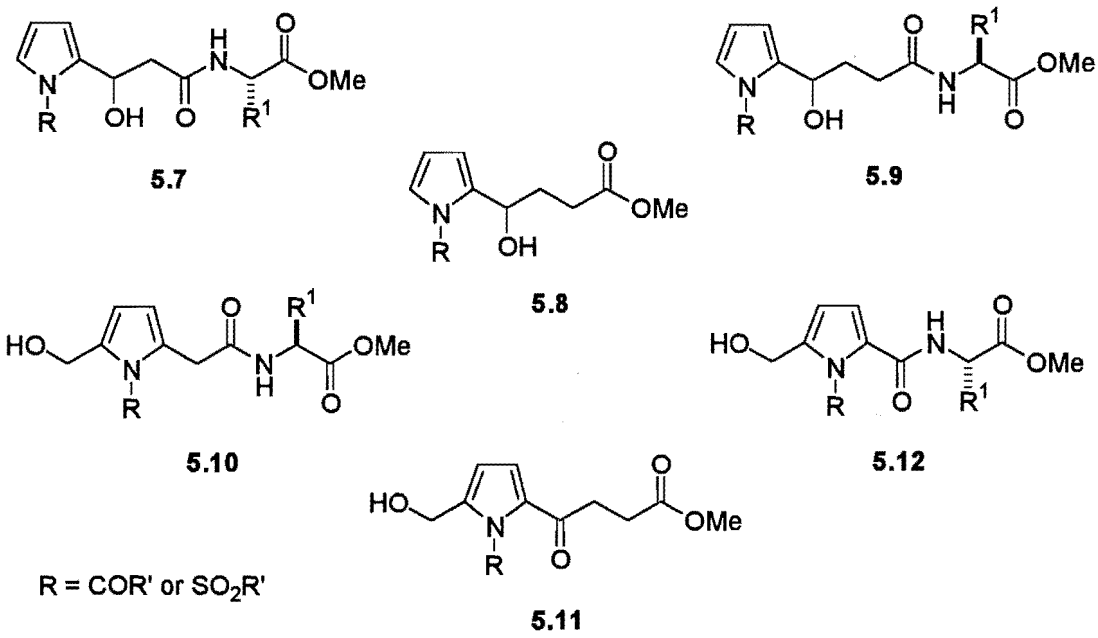
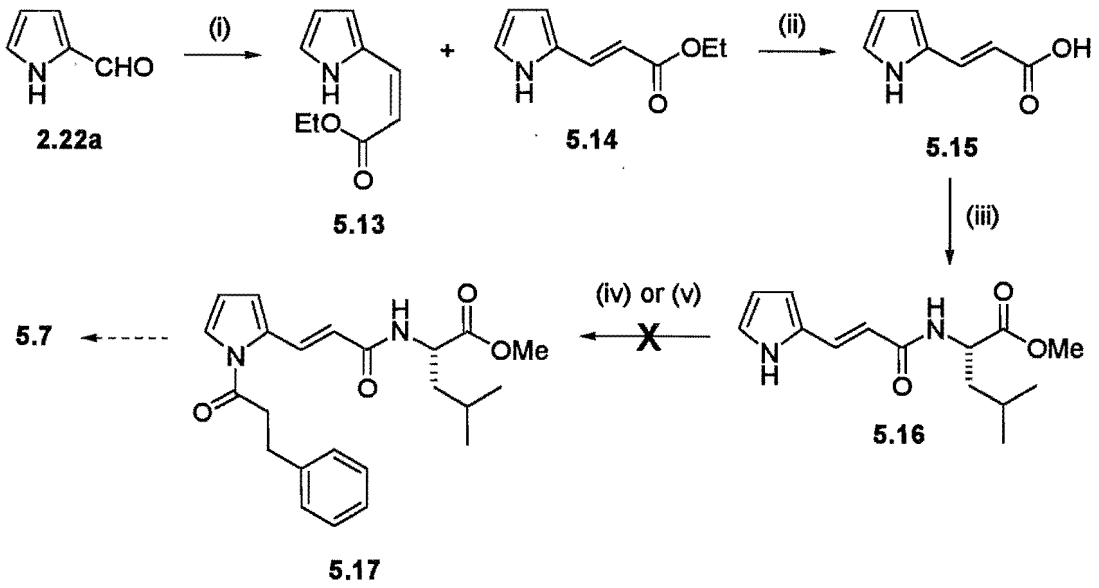



**Figure 5.4.** Key synthetic targets identified as potential peptidomimetics.

### 5.3.1. Attempted preparation of peptidomimetics of type **5.7**



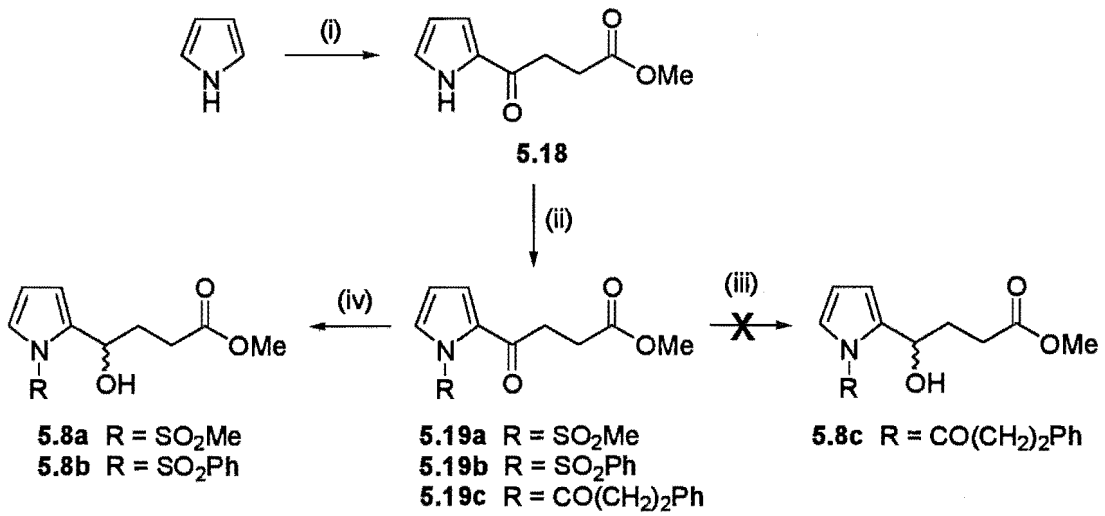

**Scheme 5.4.** *Reagents and conditions*: (i) $Ph_3P{:}CHCO_2Et$, PhH, reflux (**5.13**, 17%; **5.14** 49%); (ii) NaOH, MeOH-$H_2O$ (88%); (iii) *L*-LeuOMe.HCl, DCC, HOBt, Hünig's base (92%); (iv) NaH, THF then $Ph(CH_2)_2COCl$; (v) DMAP, Hünig's base then $Ph(CH_2)_2COCl$.

Our synthetic route to peptidomimetics of type **5.7** is shown in Scheme 5.4. The starting pyrrole-2-carboxaldehyde **2.22a** was reacted with (carboethoxy)methyl triphenylphosphorane under the Wittig conditions described by Jones and Lindner[5] to give a mixture of the alkenes **5.13** and **5.14** in 66% overall yield. The *cis*- **5.13** and *trans*-**5.14** alkenes were then separated to give the desired *trans*-isomer **5.14** in a yield of 49%. The ester of pyrrole **5.14** was hydrolysed with sodium hydroxide to give the free acid **5.15** in 88% yield, which was coupled with *L*-leucine methyl ester hydrochloride using the standard peptide coupling reagents 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt),[6] to give the pyrrole amide **5.16** in 92% yield. Subsequent attempts to prepare the desired *N*-acyl derivative **5.17** by substituting the pyrrole nitrogen of **5.16** with hydrocinnamoyl chloride, using either the sodium hydride or DMAP-

mediated acylation methodologies described in Chapter 2, gave returned starting material. $^1$H NMR spectral analysis of the crude product from these reactions failed to show any evidence of the desired *N*-acyl product **5.17**. Due to this inability to effect N-substitution of compound **5.16**, further work on peptidomimetics of type **5.7** was discontinued.

### 5.3.2. Preparation of peptidomimetics of type **5.8** and **5.9**

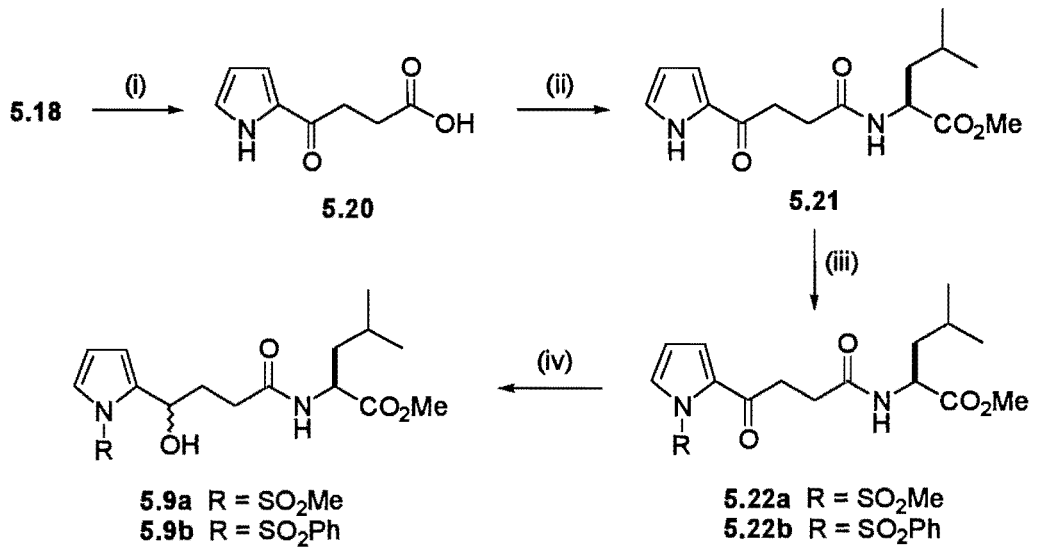
Peptidomimetics of type **5.8** and **5.9** were conveniently prepared from a common intermediate **5.18** by the synthetic routes detailed in Schemes 5.5 and 5.6. Compound **5.18** was prepared in 49% yield by acylating pyrrole with methyl succinyl chloride[7] using standard Grignard conditions.[8] The synthetic route then used to prepare compounds of type **5.8** from the intermediate **5.18** is detailed below in Scheme 5.5.




**Scheme 5.5.** *Reagents and conditions*: (i) MeMgI, $Et_2O$, reflux then $MeO_2C(CH_2)_2COCl$, reflux (49%); (ii) NaH, THF then either $MeSO_2Cl$ (**5.19a**, 86%) or $PhSO_2Cl$ (**5.19b**, 43%) or $Ph(CH_2)_2COCl$ (**5.19c**, 82%); (iii) $Zn(BH_4)_2$, $Et_2O$, 0 °C; (iv) $NaBH_4$, MeOH, 0 °C (**5.8a**, 85%), (**5.8b**, 90%).

The *N*-substituted pyrroles **5.19a-c** were readily prepared in yields of 86%, 43% and 82%, respectively, by treating the pyrrole **5.18** with sodium hydride and then reacting the resultant *N*-pyrrolyl anion with either methanesulfonyl chloride, phenylsulfonyl chloride or hydrocinnamoyl chloride. An attempted reduction of the ketone of the *N*-hydrocinnamoylpyrrole **5.19c** with zinc borohydride gave an inseparable mixture of deacylated pyrrole **5.18** and desired product **5.8c** in a ratio of 5 : 2 (by $^1$H NMR spectroscopy). Next, we turned our attention to the reduction of the more stable *N*-sulfonyl derivatives **5.19a** and **5.19b**. An attempted reduction of the ketone of the *N*-mesylpyrrole **5.19a** using zinc borohydride gave returned starting material only. However, a sodium borohydride reduction of **5.19a** gave the desired product **5.8a** in 85% yield. Similarly, a sodium borohydride reduction of the *N*-phenylsulfonylpyrrole **5.19b** gave the desired alcohol **5.8b** in 90% yield.

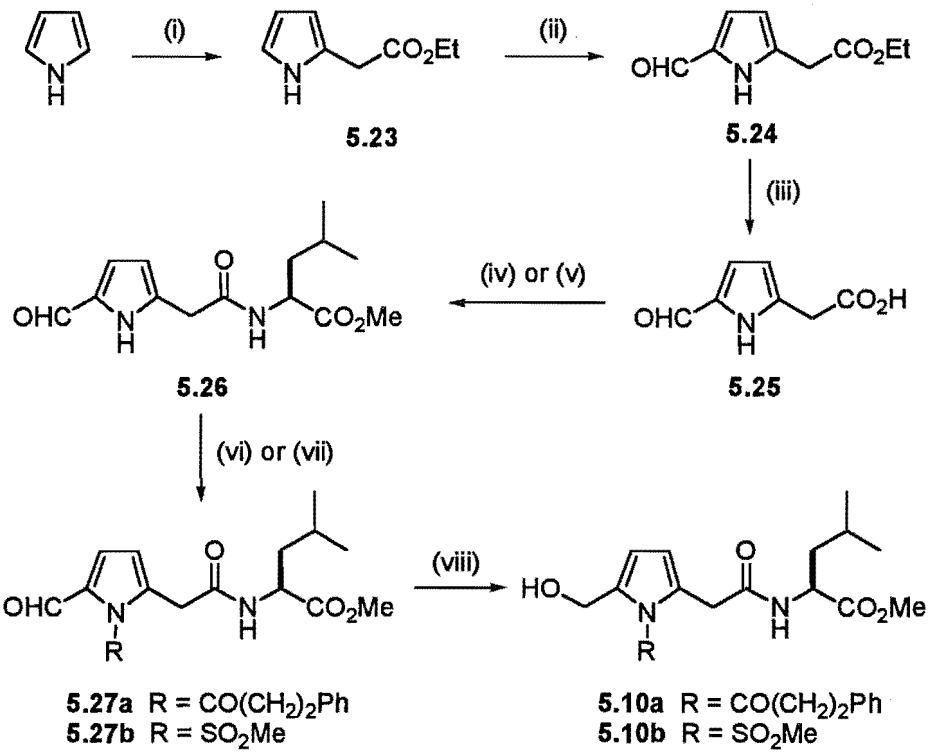
The synthetic route used to prepare peptidomimetics of type **5.9** is detailed below in Scheme 5.6.




**Scheme 5.6.** *Reagents and conditions*: (i) NaOH, MeOH-$H_2O$ (78%); (ii) *L*-LeuOMe.HCl, EDCI, HOBt, Hünig's base (93%); (iii) NaH, THF then either $MeSO_2Cl$ (**5.22a**, 50%) or $PhSO_2Cl$ (**5.22b**) (iv) $NaBH_4$, MeOH, 0 °C (**5.9a**, 89%), (**5.9b**, 38% for 2 steps).

The ester of pyrrole **5.18** was hydrolysed under basic conditions to give the free acid **5.20** in 78% yield, which was coupled with *L*-leucine methyl ester hydrochloride, using standard EDCI-coupling methodology,[9] to give the pyrrole amide **5.21** in 93% yield. N-Substitution of the pyrrole **5.21** using either methanesulfonyl chloride or phenylsulfonyl chloride, followed by sodium borohydride reduction of the ketone, then gave the desired pyrrole alcohols **5.9a** and **5.9b** in yields of 45% and 38%, respectively (2 steps).

## 5.3.3. Preparation of peptidomimetics of type **5.10**

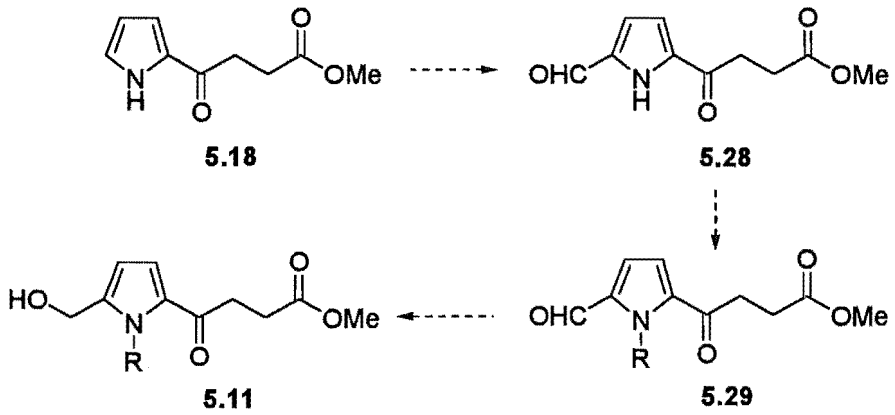



**Scheme 5.7.** *Reagents and conditions*: (i) EtMgBr, THF, –10 °C to rt then $BrCH_2CO_2Et$, –10 °C to rt then aq. $NH_4Cl$ (61%); (ii) $POCl_3$, DMF, 1,2-DCE, reflux then $NaOAc.3H_2O$, reflux (56%); (iii) NaOH, MeOH-$H_2O$ (84%); (iv) *L*-LeuOMe.HCl, EDCI, HOBt, Hünig's base (85%); (v) *L*-LeuOMe.HCl, DCC, HOBt, Hünig's base (81%); (vi) DMAP, Hünig's base then $Ph(CH_2)_2COCl$, –10 °C to rt (**5.27a**, 81%); (vii) NaH, THF then $MeSO_2Cl$, –10 °C to rt (**5.27b**, 70%); (viii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (**5.10a**, 44%), (**5.10b**, 83%).

Our synthetic route to peptidomimetics of type **5.10** is shown above in Scheme 5.7. Pyrrole was alkylated with ethyl bromoacetate under the Grignard conditions described by Schloemer *et al.*,[10] to give **5.23** in 61% yield. In this method, a 3.3 molar excess of the *N*-pyrrolylmagnesium salt was used in order to minimise the formation of polyalkylation side products. Vilsmeier-Haack formylation of **5.23** then gave the desired 2,5-disubstituted pyrrole **5.24** in 56% yield. The ester of pyrrole **5.24** was hydrolysed under basic conditions to give the free acid **5.25** in 84% yield, which was coupled with *L*-leucine methyl ester hydrochloride, using both standard EDCI and DCC-coupling methodologies, to give the pyrrole amide **5.26** in yields of 85% and 81%, respectively. Initial attempts to N-substitute the sodium salt of pyrrole **5.26** with either phenylsulfonyl chloride, β-*trans*-styrenesulfonyl chloride or 2-phenylethanesulfonyl chloride were unsuccessful, giving returned starting material and/or polymeric material. However, N-mesylation of **5.26** was subsequently achieved by employing a modified procedure in which the sodium salt of **5.26** was cooled to –10 °C during the addition of methanesulfonyl chloride. In this way, the *N*-mesylpyrrole **5.27b** was prepared in 70% yield. A similar modification proved successful in the preparation of the *N*-hydrocinnamoylpyrrole **5.27a**. Thus, addition of hydrocinnamoyl chloride to a cooled (–10 °C) dichloromethane solution of **5.26**, Hünig's base and DMAP gave the desired pyrrole **5.27a** in 81% yield. Reduction of the formyl group of **5.27b** with zinc borohydride then gave the desired hydroxymethylpyrrole **5.10b** in 83% yield. A similar zinc borohydride reduction of **5.27a** gave the desired hydroxymethylpyrrole **5.10a** in 44% yield. However, the resulting product **5.10a** was unstable and was observed to slowly decompose at 0 °C.

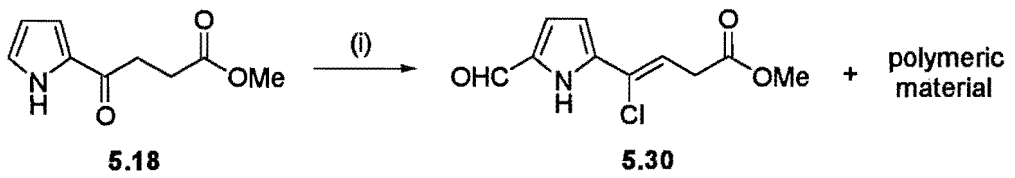
### 5.3.4. Attempted preparation of peptidomimetics of type **5.11**

Our proposed synthetic route to peptidomimetics of type **5.11** is shown below in Scheme 5.8.



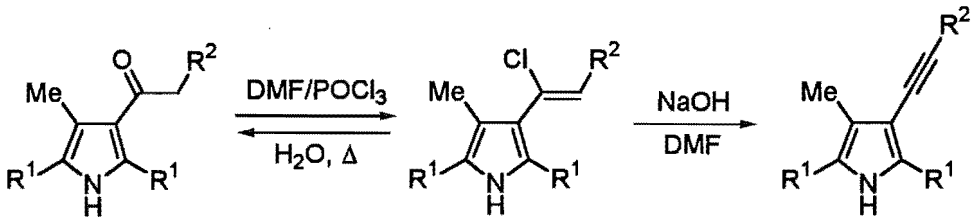

**Scheme 5.8.** Proposed synthetic route to peptidomimetics of type **5.11**.

An initial attempt to prepare **5.28** by Vilsmeier-Haack formylation of the corresponding pyrrole **5.18**, prepared as detailed in Scheme 5.5, proved unsuccessful. Instead, the α-chlorovinylpyrrole **5.30** was obtained in low yield (13%), along with a considerable amount of polymeric material (Scheme 5.9).




**Scheme 5.9.** *Reagents and conditions*: (i) $POCl_3$, DMF, 1,2-DCE, reflux then $NaOAc.3H_2O$, reflux (13%).

An analogous conversion of fully ring-substituted 3-acylpyrroles into α-chlorovinylpyrroles under Vilsmeier-Haack conditions has previously been reported by Mironov *et al.*[11] The resulting α-chlorovinylpyrroles were then readily hydrolysed under aqueous conditions to regenerate the starting 3-acylpyrroles (Scheme 5.10). However, when the more strongly basic conditions of sodium hydroxide in *N,N*-dimethylformamide were employed in this hydrolysis, 3-alkynylpyrroles were formed.

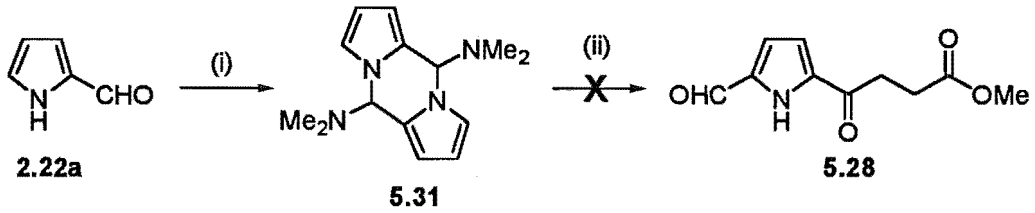



$R^1$ = Me, $CO_2Et$; $R^2$ = H, Me, Ph

**Scheme 5.10.** Analogous α-chlorovinylpyrrole formation (Mironov *et al.*).

A subsequent attempt was then made to prepare **5.28** using anion chemistry by employing the methodology described by Muchowski and Hess.[12] This method involves treating the azafulvene dimer **5.31** (Scheme 5.11) with a suitable organolithium reagent to give a dilithiated species, which after reaction with an electrophile and hydrolysis, then affords a 5-substituted pyrrole-2-carboxaldehyde. A diverse range of electrophiles have been utilised in this sequence to provide a wide variety of 5-substituted pyrrole-2-carboxaldehydes, including an example of a 5-acyl pyrrole-2-carboxaldehyde.[12]

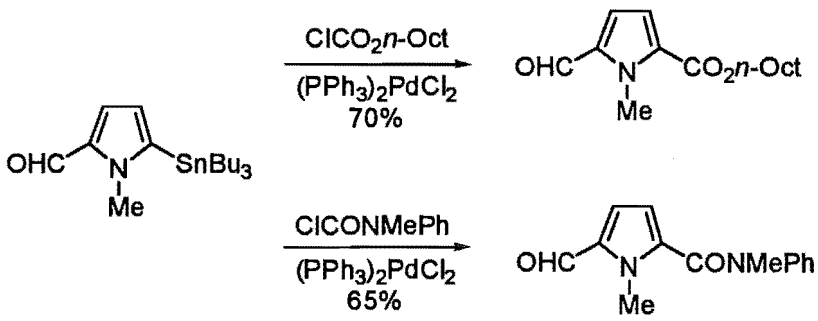
The azafulvene dimer **5.31** was readily prepared in a yield of 76% by treating pyrrole-2-carboxaldehyde **2.22a** with a solution of 40% aqueous dimethylamine.[12] A solution of the dimer **5.31** in tetrahydrofuran at –15 °C was treated with *tert*-butyl lithium (2.5 equivalents) to give a dilithiated species, which was then quenched at –78 °C by the addition of 3-carbomethoxypropionyl-*N*-methoxy-*N*-methylamide.[13] However, subsequent hydrolysis of the reaction mixture gave returned pyrrole-2-carboxaldehyde




**Scheme 5.11.** *Reagents and conditions*: (i) 40% aq. $Me_2NH$ (76%); (ii) *t*-BuLi, –15 °C to 0 °C then $MeO_2C(CH_2)_2CON(OMe)Me$, –78 °C to rt then aq. NaOAc, reflux.

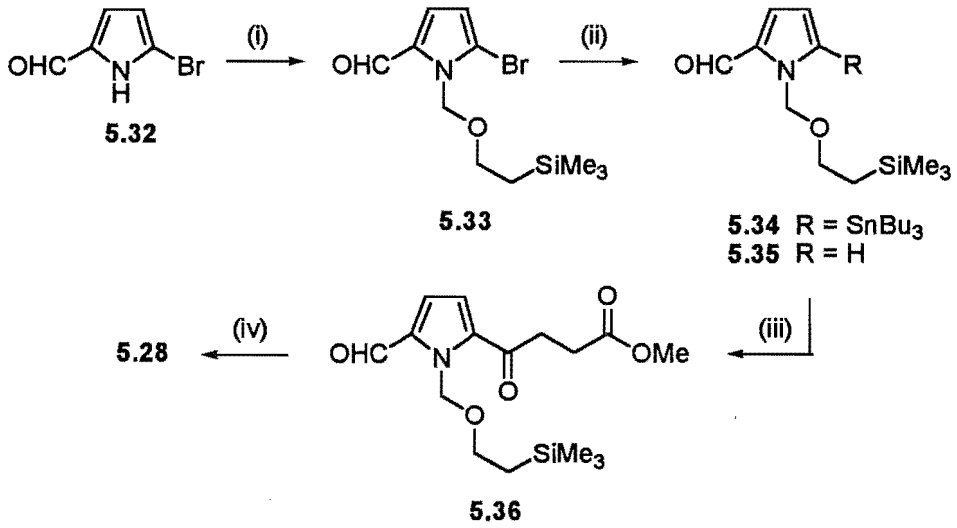
**2.22a** only (68% recovery). At this stage it remains uncertain why none of the desired 2-acylated product **5.28** was isolated from this reaction sequence.

Due to the inability to prepare the desired compound **5.28** by the methods described above, we decided to investigate an alternative strategy. It was thought that a Stille coupling of a stannylpyrrole with an acid chloride might be a mild and versatile route by which to introduce the second acyl substituent onto the pyrrole ring. This was in light of the work by Jousseaume *et al.*[14] which had reported that the palladium-catalysed cross-coupling reactions between *N*-methyl-5-tributylstannylpyrrole-2-carboxaldehyde and chloroformates or carbamoyl chlorides gave the corresponding formylpyrrole esters and amides in good yields (65 – 70%), and without the need for protection of the formyl group (see Scheme 5.12).




**Scheme 5.12.** Palladium-catalysed cross-coupling reaction of stannylpyrroles (Jousseaume *et al.*).

However, these previous Stille couplings had been carried out with a stannylpyrrole *N*-substituted with a methyl group. Such a group would be undesirable in our current synthesis as it would be difficult to remove at the end of the reaction sequence. We therefore chose the 2-(trimethylsilyl)ethoxymethyl (SEM) group[15] as an alternative, as it would be both stable to the reaction conditions, and would also be easily removed at the end of the reaction sequence. Furthermore, a SEM group is known to direct substitution at the α-position of pyrrole due to the formation of a stabilised chelated species upon lithiation.[15] Our initial synthetic route to compound **5.28** utilising this SEM-protection of the pyrrole nitrogen is shown in Scheme 5.13.



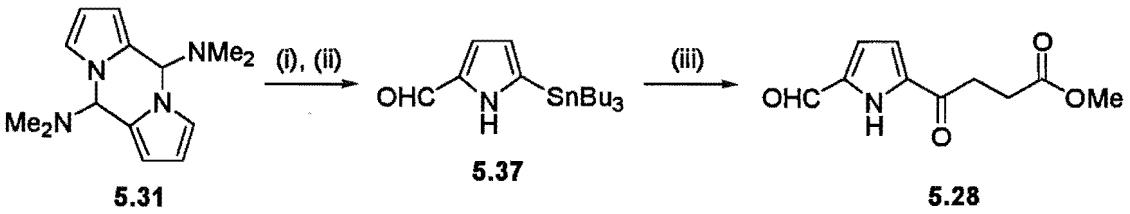

**Scheme 5.13.** *Reagents and conditions*: (i) NaH, DMF then SEM-Cl (89%); (ii) Morpholine, *n*-BuLi, THF, –78 °C then *t*-BuLi then *n*-$Bu_3SnCl$, –78 °C to rt (**5.34**, 60%; **5.35**, 25%); (iii) $MeO_2C(CH_2)_2COCl$, $(Ph_3P)_2PdCl_2$, PhMe, reflux (74%); (iv) $BF_3.OEt_2$, $CH_2Cl_2$, 0 °C then aq. NaOAc, reflux (75%).

The starting 5-bromopyrrole-2-carboxaldehyde **5.32** was prepared by the indirect method described by Bray and Muchowski.[16] The *N*-protected pyrrole **5.33** was then prepared in 89% yield by treating **5.32** with sodium hydride in *N,N*-dimethylformamide, and reacting the resultant *N*-pyrrolyl anion with 2-(trimethylsilyl)ethoxymethyl chloride

(SEM-Cl). In the key stannylation step, *in situ* protection of the formyl group of **5.33** by treatment with lithium morpholide,[17] followed by bromine-lithium exchange at –78 °C using *tert*-butyl lithium (2.2 equivalents) and quenching of the resultant lithiated species at –78 °C with tri-*n*-butylstannyl chloride, gave a mixture of the desired stannylpyrrole **5.34** and debrominated starting material **5.35** in a ratio of 5 : 2 (by $^1$H NMR spectroscopy). These compounds were separated by flash chromatography on silica to give **5.34** and **5.35** in yields of 60% and 25%, respectively. The stannylpyrrole **5.34** was coupled with methyl succinyl chloride using bis(triphenylphosphine)palladium(II) dichloride in refluxing toluene to give the 5-acylpyrrole-2-carboxaldehyde **5.36** in 74% yield. In the final step, deprotection of the pyrrole nitrogen was readily achieved by treating **5.36** with boron trifluoride diethyl etherate at 0 °C, followed by heating the crude products at reflux with aqueous sodium acetate,[15a] to give the desired product **5.28** in 75% yield.

Having successfully prepared **5.28** through a Stille coupling of an *N*-protected stannylpyrrole, we then set out to investigate whether we could prepare **5.28** through a similar Stille coupling procedure, but without having to protect the pyrrole nitrogen. All previously reported Stille couplings of stannylpyrroles have employed *N*-substitution for protection and/or stability reasons.[14,18] However, it has recently been reported by Fukuyama *et al.*[19] that an *N*-unprotected stannylindole was able to undergo a Stille coupling in good yield (71%). This suggested to us that a Stille coupling of an *N*-unprotected stannylpyrrole therefore ought to be possible too. Our synthetic route to compound **5.28**, without protection of the pyrrole nitrogen, is shown in Scheme 5.14.

The key starting *N*-unsubstituted stannylpyrrole **5.37** was prepared from the azafulvene dimer **5.31** by a modified method to that described by Denat *et al.*[20] In this method, a solution of the dimer **5.31** in tetrahydrofuran at –15 °C was treated with *tert*-butyl lithium (2.2 equivalents) to give a dilithiated species, which was then quenched at –78 °C by the addition of tri-*n*-butylstannyl chloride. Subsequent hydrolysis of this reaction mixture by heating the crude products at reflux with aqueous sodium acetate for

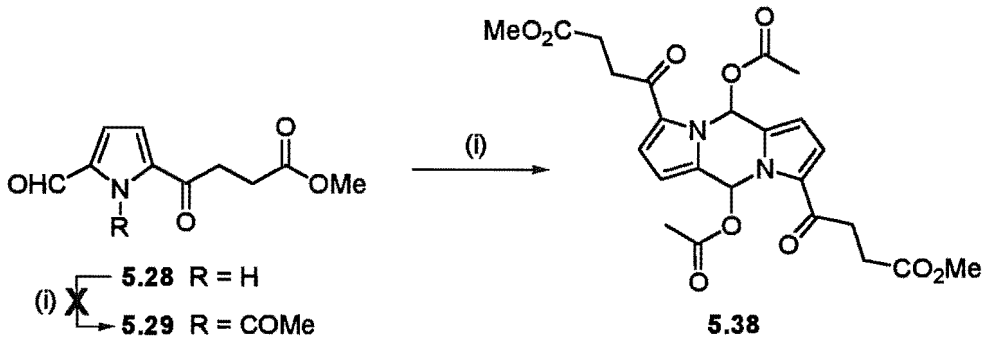



**5.31** **5.37** **5.28**

**Scheme 5.14.** *Reagent and conditions*: (i) *t*-BuLi, THF, –15 °C to 0 °C then *n*-$Bu_3SnCl$, –78 °C to rt; (ii) aq. NaOAc, THF, reflux, 5 days (37% for 2 steps); (iii) $MeO_2C(CH_2)_2COCl$, $(Ph_3P)_2PdCl_2$, PhMe, reflux then KF, MeCN, rt (78%).

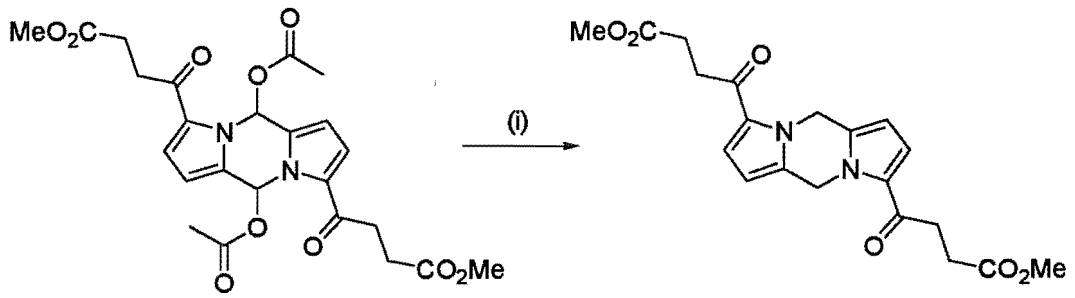
5 days then gave the stannylpyrrole **5.37** in 37% yield (2 steps). The stannylpyrrole **5.37** was coupled with methyl succinyl chloride using bis(triphenylphosphine)palladium(II) dichloride in refluxing toluene to give the desired compound **5.28** in 78% yield. The acylation occurred exclusively at the 5-position under these conditions, with no *N*-acylated pyrrole side-products being detected by $^1$H NMR spectroscopy. To the best of our knowledge, this is the first example of a Stille coupling of an *N*-unsubstituted pyrrole. This method therefore allows for a fast and efficient synthesis of a range of 5-acylpyrrole-2-carboxaldehydes from a single key precursor, and without the need to protect either the formyl group or the pyrrole nitrogen (see Chapter 7 for an application of this method in the preparation of a marine natural product).

Having successfully prepared the intermediate 5-acylpyrrole-2-carboxaldehyde **5.28** by the methods described above, an attempt was then made to prepare the desired *N*-acyl derivative **5.29** (see Scheme 5.15). Consequently, compound **5.28** with treated with sodium hydride, and the resulting pyrrolyl anion was reacted with acetyl chloride (Scheme 5.15). Work up of the reaction mixture and purification of the residue by flash chromatography on silica gave a single pyrrole-based compound, which was tentatively assigned as the 6-acetyloxy-1-azafulvene dimer **5.38** (89% yield) on the basis of the NMR and mass spectral data discussed below.



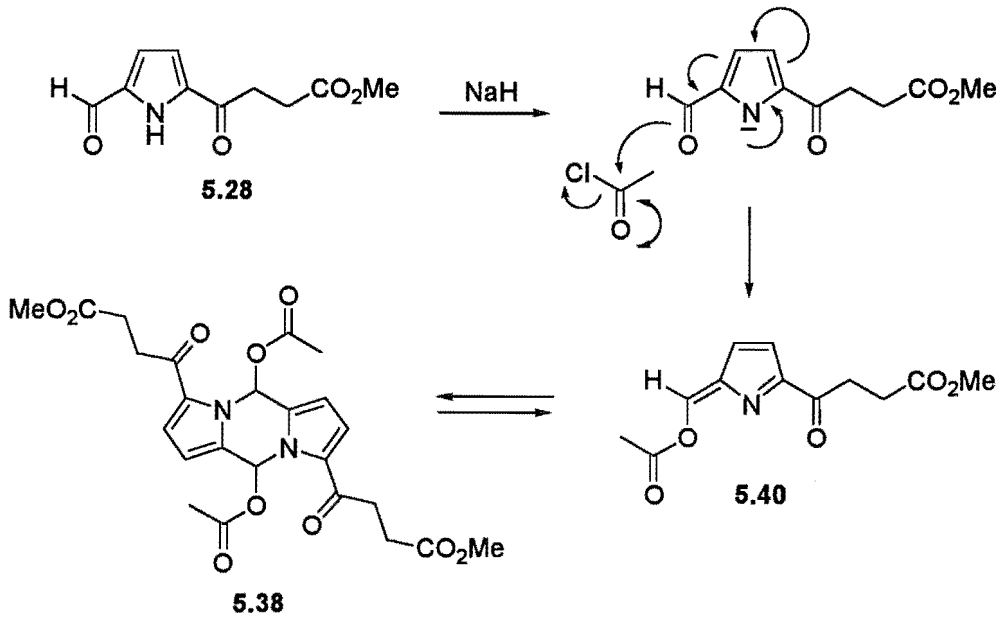

**Scheme 5.15.** *Reagents and conditions*: (i) NaH, THF then MeCOCl (89%).

The $^1$H NMR spectrum of the purified product was inconsistent with the desired *N*-acetylpyrrole **5.29** (Scheme 5.15), since a proton signal was not observed for the formyl group (expected at approximately δ 10.3). However, the $^1$H NMR spectrum did indicate that *N*-substitution had occurred on pyrrole since no nitrogen proton could be observed. Furthermore, the $^1$H and $^{13}$C NMR spectra indicated the presence of an acetate group in the molecule, due to a three proton signal at δ 2.06 and carbon signals at δ 20.8 and 169.4. The dimeric nature of **5.38** was then proposed on the basis of a high resolution mass spectrum which was consistent with the structural formulae $C_{22}H_{23}N_2O_8$ (MH-$CH_3CO_2H$). To confirm our structural assignment, and also to determine the relative stereochemistry at the 6-acetyloxy positions (*ie syn-* or *anti*-configuration), attempts were made to grow crystals of **5.38** suitable for X-ray crystallographic analysis. However, despite numerous crystallisation attempts from various solvent systems (*eg* ethyl acetate/petroleum ether, chloroform/petroleum ether and acetone/petroleum ether) we were unable to grow crystals suitable for such an analysis. Nonetheless, confirmation of our proposed dimeric structure for **5.38** was obtained by the subsequent conversion of **5.38**, via a reductive cleavage reaction,[21] to the reduced pyrazine **5.39** (16% yield) which was then fully characterised (Scheme 5.16).




**Scheme 5.16.** *Reagents and conditions*: (i) $Zn(BH_4)_2$, $Me_3SiCl$, $Et_2O$, 0 °C (16%).

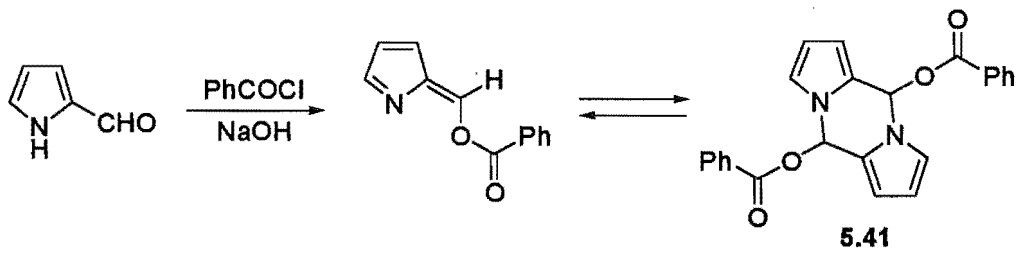
A possible mechanism for the formation of the 6-acetyloxy-1-azafulvene dimer **5.38** is shown below in Scheme 5.17.




**Scheme 5.17.** Possible mechanism for the formation of azafulvene dimer **5.38**.

Deprotonation of the starting 5-acylpyrrole-2-carboxaldehyde **5.28** generates the corresponding pyrrolyl anion which can then react with acetyl chloride through the oxygen of the formyl group, as shown in Scheme 5.17. The resulting *O*-acylazafulvene

intermediate **5.40** can then undergo facile dimerisation to give the 6-acetyloxy-1-azafulvene dimer **5.38**. An analogous *O*-acylation/dimerisation mechanism has been proposed to explain the formation of the related 6-benzoyloxy-1-azafulvene dimer **5.41** upon benzoylation of pyrrole-2-carboxaldehyde (Scheme 5.18).[22]

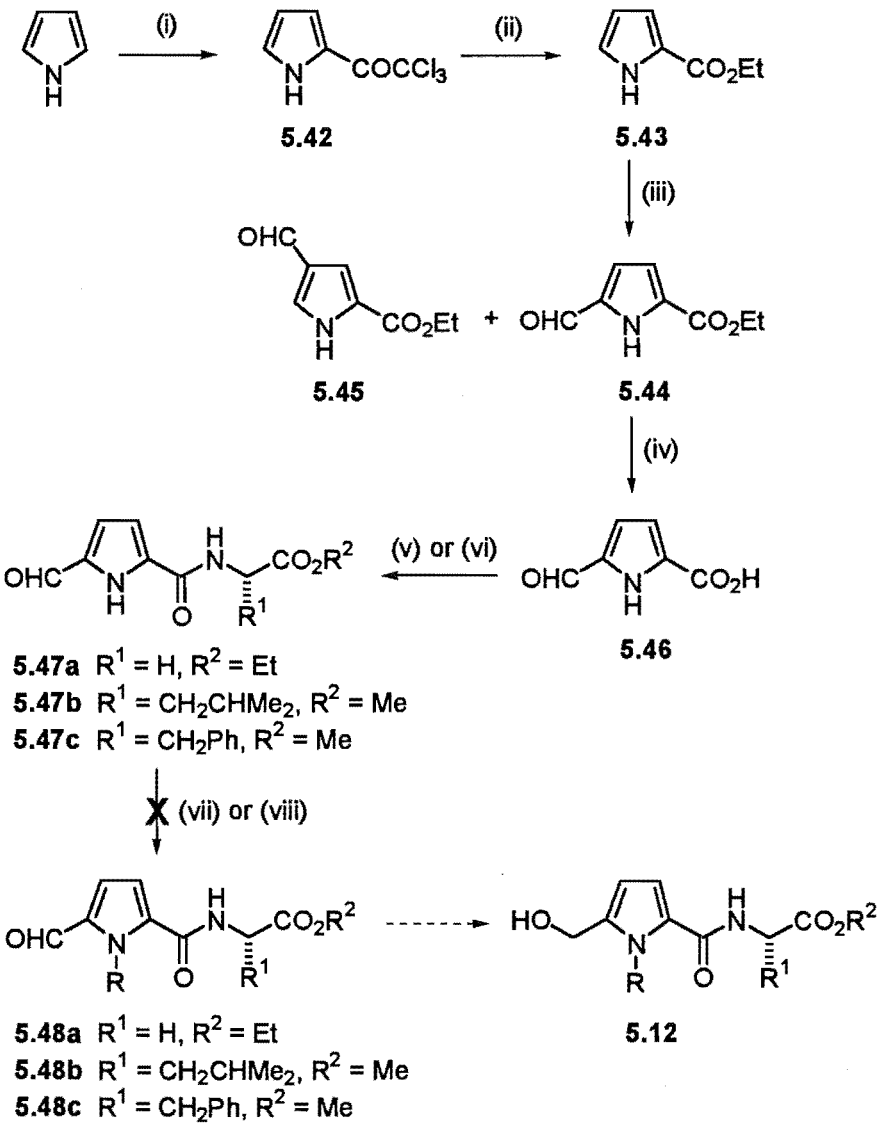



**Scheme 5.18.** Formation of the 6-benzoyloxy-1-azafulvene dimer **5.41** (Treibs *et al.*).

Due to the inability to effect the desired N-substitution of compound **5.28**, further work on peptidomimetics of type **5.11** was discontinued.

## 5.3.5. Attempted preparation of peptidomimetics of type **5.12**

Our synthetic route to peptidomimetics of type **5.12** is shown in Scheme 5.19.




**Scheme 5.19.** *Reagents and conditions*: (i) $CCl_3COCl$, $Et_2O$ (81%); (ii) EtOH, Na (98%); (iii) $POCl_3$, DMF, 1,2-DCE, reflux then $NaOAc.3H_2O$, reflux (**5.44**, 67%; **5.45**, 15%); (iv) KOH, $H_2O$, 40 – 50 °C (82%); (v) EDCI, HOBt, Hünig's base and either GlyOEt.HCl (**5.47a**, 70%) or *L*-LeuOMe.HCl (**5.47b**, 90%) or *L*-PheOMe.HCl (**5.47c**, 91%); (vi) *L*-LeuOMe.HCl, DCC, HOBt, Hünig's base (**5.47b**, 89%); (vii) DMAP, Hünig's base then RCOCl; (viii) NaH, THF, then RCOCl.

Pyrrole was acylated with trichloroacetyl chloride to give **5.42**, which was then treated with sodium ethoxide in ethanol to give ethyl pyrrole-2-carboxylate **5.43** in 79% yield (2 steps).[8] Vilsmeier-Haack formylation of **5.43** then afforded a mixture of the formylpyrrole isomers **5.44** and **5.45** in 82% overall yield. The 2,5- **5.44** and 2,4- **5.45** disubstituted pyrroles were separated to give the desired pyrrole **5.44** in 67% yield. Next, the ester of pyrrole **5.44** was hydrolysed under basic conditions to give the free acid **5.46** in 82% yield, which was coupled with either glycine ethyl ester hydrochloride, *L*-leucine methyl ester hydrochloride or *L*-phenylalanine methyl ester hydrochloride using standard EDCI-peptide coupling methodology to give the key 5-formylpyrrole-2-carboxamides **5.47a-c** in yields of 70%, 90% and 91%, respectively. Compound **5.47b** was also prepared in high yield (89%) using standard DCC-peptide coupling methodology.

Subsequent attempts to prepare the desired *N*-acyl derivatives **5.48a-c** by N-substitution of the corresponding 5-formylpyrrole-2-carboxamides **5.47a-c**, using either the DMAP or sodium hydride acylation methodologies described in Chapter 2, were unsuccessful and instead led to the formation of the unexpected heterocyclic products **6.1a-c** and **6.6a-c**, respectively (see Figure 5.5). For further discussion on the preparation and properties of these unexpected heterocyclic products see Chapter 6. Due to this inability to effect the desired N-substitution of compounds **5.47a-c**, further work on peptidomimetics of type **5.12** was discontinued.

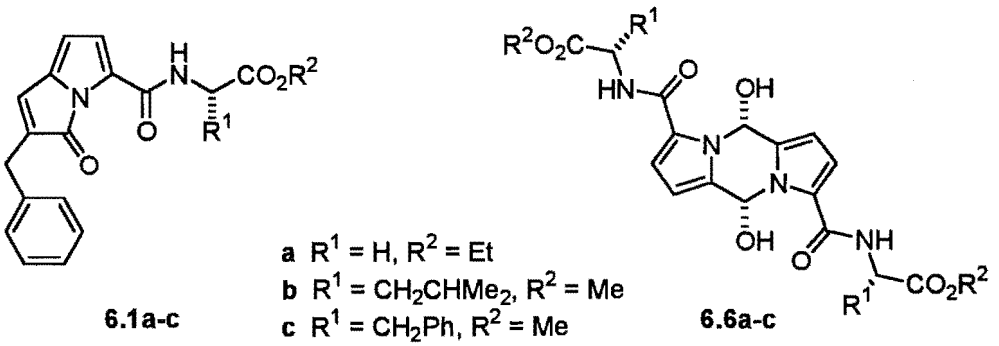



**Figure 5.5.** Unexpected products obtained from N-substitution of pyrroles **5.47a-c**.

## 5.3.6. α-Chymotrypsin assay of pyrrole-based peptidomimetics

The peptide-based pyrroles **5.8a-b**, **5.9a-b** and **5.10b** (Figure 5.6) were assayed as potential inhibitors of α-chymotrypsin by the colorimetric method outlined in Section 5.2. The results of this α-chymotrypsin assay are presented in Table 5.3.

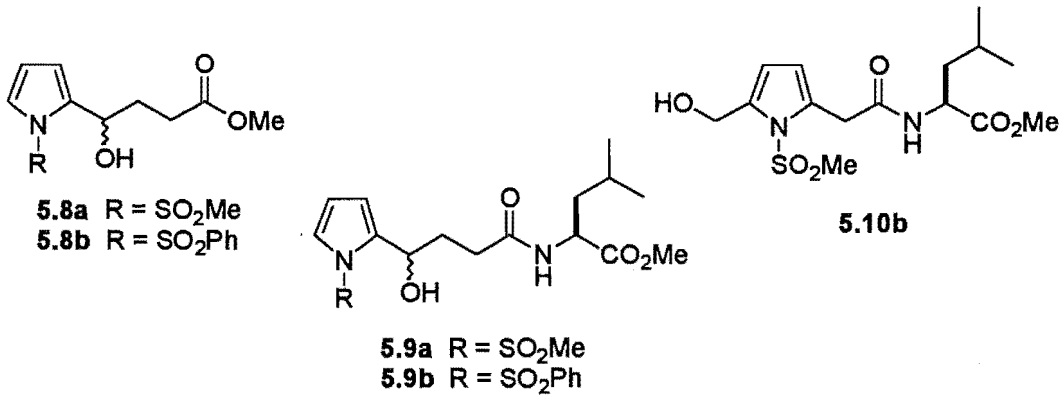



**Figure 5.6.** Peptide-based pyrroles assayed as potential inhibitors of α-chymotrypsin.

| Compound | % Inhibition | | |
|---|---|---|---|
| | 1250 μg $mL^{-1}$ | 125 μg $mL^{-1}$ | 12.5 μg $mL^{-1}$ |
| **5.8a** | 10 | 5 | 0 |
| **5.8b** | 25 | 10 | 0 |
| **5.9a** | 0 | 0 | 0 |
| **5.9b** | 20 | 15 | 10 |
| **5.10b** | 10 | 5 | 0 |

**Table 5.3.** Results of α-chymotrypsin assay (results rounded to nearest 5%).

The results detailed in Table 5.3 indicate that the extended pyrrole derivatives **5.8a-b**, **5.9b** and **5.10b** developed in this work were modest inhibitors of α-chymotrypsin. Of the 2-extended compounds tested, the *N*-phenylsulfonyl derivatives

**5.8b** and **5.9b** were found to show significantly greater enzyme inhibition activity than the corresponding *N*-mesyl derivatives **5.8a** and **5.9a**. As previously discussed, this was attributed to the mesyl group being a poor substituent for recognition by α-chymotrypsin. A further point to note from the results detailed in Table 5.3, was that the more extended peptide-like examples **5.9a** and **5.9b** failed to show any increase in the enzyme inhibition activity compared to the more simple examples **5.8a** and **5.8b**. In fact, in the case of the extended *N*-mesyl example **5.9a**, the increase in chain length led to a complete loss of enzyme inhibition activity. This may have been due to either poor enzyme recognition of the inhibitor molecule (*ie* leucine not the optimal residue at the $P_3'$ subsite for binding in the enzyme), or due to steric congestion within the enzyme active site caused by this increase in chain length (*ie* steric interactions preventing the efficient binding of the inhibitor molecule in the enzyme).

The 2,5-extended derivative **5.10b** also failed to show any significant inhibition activity against α-chymotrypsin. This again was most likely due to poor enzyme recognition of the *N*-mesyl substituent. A final point to note from the results detailed in Table 5.3 was that of the peptide-based pyrroles tested in this current study, none proved to be better inhibitors than previously discussed examples.

## 5.4. Summary and future work

We have investigated the application of *N*-substituted hydroxymethylpyrroles as mechanism-based inhibitors of serine proteases. In preliminary studies to determine the optimal pyrrole *N*-substituent and leaving group for enzyme inhibition, we found that an *N*-arylsulfonyl group was able to promote the inhibition of α-chymotrypsin. Up to this time only *N*-acyl substituted hydroxymethylpyrroles had been investigated in this role. Furthermore, we have found that a hydroxyl group at the 2-methyl position of pyrrole was the optimum leaving group for enzyme inhibition.

Subsequent work then focussed on identifying and developing potential lead compounds as possible pyrrole-based peptidomimetics. As a result, we have investigated a number of alternate methodologies to incorporate the hydroxymethylpyrrole-moiety into an extended peptide-like sequence. During the attempted preparation of many of these compounds, however, problems were encountered in the pyrrole N-substitution step, with either N-substitution failing to occur, or conversely, resulting in the formation of unexpected heterocyclic side-products. Of the compounds that were prepared and assayed for enzyme inhibition activity in this current work, none proved to be better inhibitors than previously discussed examples.

Future work in this area could entail the further development of some of the lead compounds identified in this work as mechanism-based inhibitors of serine proteases. This could potentially lead to the preparation of derivatives which display more selective and efficient enzyme inhibition activity. Further work is also required to develop a better understanding of the exact mechanisms involved in enzyme inhibition for this class of mechanism-based inhibitor.

## 5.5. References for chapter five


1 (a) Hörl, W. H.; Heidland, A. Eds. *Proteases II: Potential Role in Health and Disease.* In *Advances in Experimental Medicine and Biology.* Plenum Press: New York, 1988, Vol 240; (b) Groutas, W. C. *Med. Res. Rev.* **1987**, *7*, 227.

2 (a) Abell, A. D.; Litten, J. C. *Tetrahedron Lett.* **1992**, *33*, 3005; (b) Abell, A. D.; Litten, J. C. *Aust. J. Chem.* **1993**, *46*, 1473.

3 Litten, J. C. PhD Thesis, University of Canterbury, Christchurch, New Zealand, 1992.

4 (a) Cannell, R. J. P.; Kellam, S. J.; Owsianka, A. M.; Walker, J. M. *Planta Med.* **1988**, *54*, 10; (b) Cannell, R. J. P.; Kellam, S. J.; Owsianka, A. M.; Walker, J. M. *J. Gen. Microbiol.* **1987**, *133*, 1701.

5 Jones, R. A.; Lindner, J. A. *Aust. J. Chem.* **1965**, *18*, 875.

6 Bodanszky, M. *Principles of Peptide Synthesis.* Springer-Verlag: Berlin, Heidelberg, 1984.

7 Cason, J. *Org. Synth., Coll. Vol.* **1955**, *3*, 169.

8 Wallace, D. M.; Leung, S. H.; Senge, M. O.; Smith, K. M. *J. Org. Chem.* **1993**, *58*, 7245.

9 Tian, Z. –Q.; Brown, B. B.; Mack, D. P.; Hutton, C. A.; Bartlett, P. A. *J. Org. Chem.* **1997**, *62*, 514.

10 Schloemer, G. C.; Greenhouse, R.; Muchowski, J. M. *J. Org. Chem.* **1994**, *59*, 5230.

11 Mironov, A. F.; Kozhich, D. T.; Vasilevsky, V. I.; Evstigneeva, R. P. *Synthesis* **1979**, 533.

12 Muchowski, J. M.; Hess, P. *Tetrahedron Lett.* **1988**, *29*, 777.

13 Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.

14 Jousseaume, B.; Kwon, H.; Verlhac, J. –B.; Denat, F.; Dubac, J. *Synlett.* **1993**, 117.

15 (a) Muchowski, J. M.; Solas, D. R. *J. Org. Chem.* **1984**, *49*, 203; (b) Edwards, M. P.; Doherty, A. M.; Ley, S. V.; Organ, H. M. *Tetrahedron* **1986**, *42*, 3723.

16 Bray, B. L.; Muchowski, J. M. *Can. J. Chem.* **1990**, *68*, 1305.

17 For further examples see: (a) Lee, G. C. M.; Holmes, J. M.; Harcourt, D. A.; Garst, M. E. *J. Org. Chem.* **1992**, *57*, 3126; (b) Denat, F.; Gaspard-Iloughmane, H.; Dubac, J. *Synthesis* **1992**, 954.

18 For examples see: (a) Martina, S.; Enkelmann, V.; Wegner, G.; Schlüter, A. –D. *Synthesis* **1991**, 613; (b) Martina, S.; Enkelmann, V.; Schlüter, A. –D.; Wegner, G. *Synth. Met.* **1991**, *41*, 403; (c) Dijkstra, H. P.; ten Have, R.; van Leusen, A. M. *J. Org. Chem.* **1998**, *63*, 5332; (d) Wang, J.; Scott, A. I. *Tetrahedron Lett.* **1996**, *37*, 3247.

19 Fukuyama, T.; Chen, X.; Peng, G. *J. Am. Chem. Soc.* **1994**, *116*, 3127.

20 Denat, F.; Gaspard-Iloughmane, H.; Dubac, J. *J. Organomet. Chem.* **1992**, *423*, 173.

21 Kotsuki, H.; Ushio, Y.; Yoshimura, N.; Ochi, M. *J. Org. Chem.* **1987**, *52*, 2594.

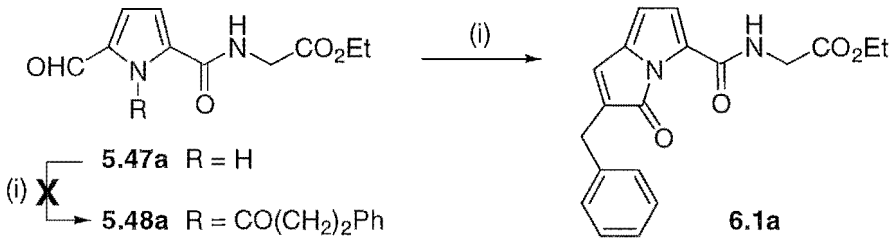
22 Treibs, A.; Kolm, H. G. *Liebigs Ann. Chem.* **1957**, *606*, 166.

# CHAPTER SIX

# FORMATION OF UNEXPECTED HETEROCYCLIC PRODUCTS DURING *N*-ACYLATION

## 6.1. Attempted N-acylation of 5.47 using DMAP methodology: Pyrrolizin-3-one formation

An attempt was made to *N*-acylate the 5-formylpyrrole-2-carboxamide **5.47a** using the DMAP acylation methodology described in Chapter 2. Accordingly, a dichloromethane solution of **5.47a**, Hünig's base (2.1 equivalents) and DMAP (0.1 equivalent) was treated with hydrocinnamoyl chloride (2.1 equivalents). The resultant yellow solution was then stirred for 24 hours, during which time the solution changed to a bright orange colour. Work up of the reaction mixture and purification of the residue by flash chromatography on silica gave a single orange coloured pyrrole-based compound, which was subsequently identified as the pyrrolizin-3-one **6.1a** (58% yield) (Scheme 6.1).




**Scheme 6.1.** *Reagents and conditions*: (i) DMAP, Hünigs base, $CH_2Cl_2$ then $Ph(CH_2)_2COCl$ (58%).

The $^1H$ NMR spectrum of the purified product (Figure 6.1) was inconsistent with the desired *N*-hydrocinnamoyl pyrrole **5.48a** (see Scheme 6.1), since resonances were not observed for the formyl proton (expected at approximately δ 10.3) and for a methylene group of the hydrocinnamate (expected at approximately δ 3.2). Nonetheless, the $^1H$ NMR spectrum did indicate that *N*-acylation had occurred on pyrrole due to the disappearance of the signal for the pyrrole nitrogen proton, while a five proton aromatic signal at δ 7.23 – 7.37 indicated the presence of the hydrocinnamoyl group.

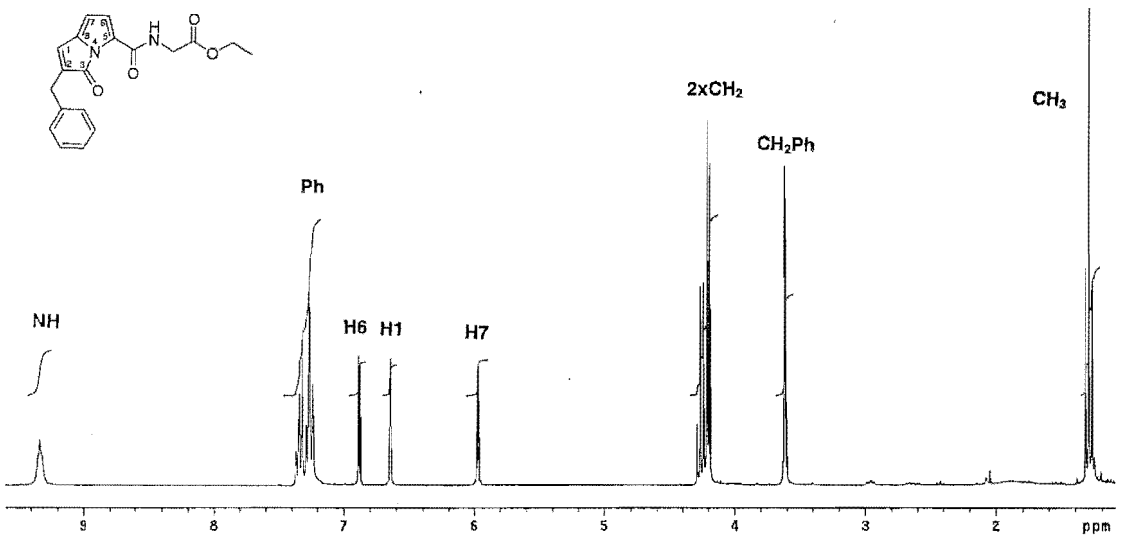



**Figure 6.1.** $^{1}H$ NMR spectrum ($CDCl_3$) of the pyrrolizin-3-one **6.1a**.

The structure of the pyrrolizin-3-one **6.1a** shown in Figure 6.1 was then proposed through the use of one and two dimensional NMR spectral techniques. Key HMBC correlations which enabled the structural assignment were identified between the proton resonance at δ 6.65 (*ie* pyrrolizin-3-one H1, see Figure 6.1 for pyrrolizin-3-one numbering scheme) and the carbon resonances at δ 31.9, 167.8 and 139.7 (*ie* $\mathbf{CH_2}$Ph, pyrrolizin-3-one C3 and pyrrolizin-3-one C8, respectively), and also between the proton resonance at δ 3.62 (*ie* $\mathbf{CH_2}$Ph) and the carbon resonances at δ 137.0, 136.8, 132.3 and 167.8 (*ie ipso*-Ph, pyrrolizin-3-one C2, pyrrolizin-3-one C1 and pyrrolizin-3-one C3, respectively). These key HMBC correlations suggested a second ring was present in the structure. A high resolution mass measurement was also consistent with the structural formulae $C_{19}H_{18}N_2O_4$ and hence the cyclic structure. Furthermore, the ultraviolet spectrum in methanol showed $\lambda_{max}$ at 242, 338 and 423 nm, which indicated a conjugated aromatic system.

Subsequent conformation of the structure of the pyrrolizin-3-one **6.1a** was obtained by a single crystal X-ray analysis. Compound **6.1a** was found to crystallise in

the monoclinic space group $P2_1/c$ with two independent molecules in the asymmetric unit (designated **6.1a** and **6.1a'**), each of which have similar conformations. A perspective drawing of **6.1a** and **6.1a'**, with atomic labelling, is presented in Figure 6.2.

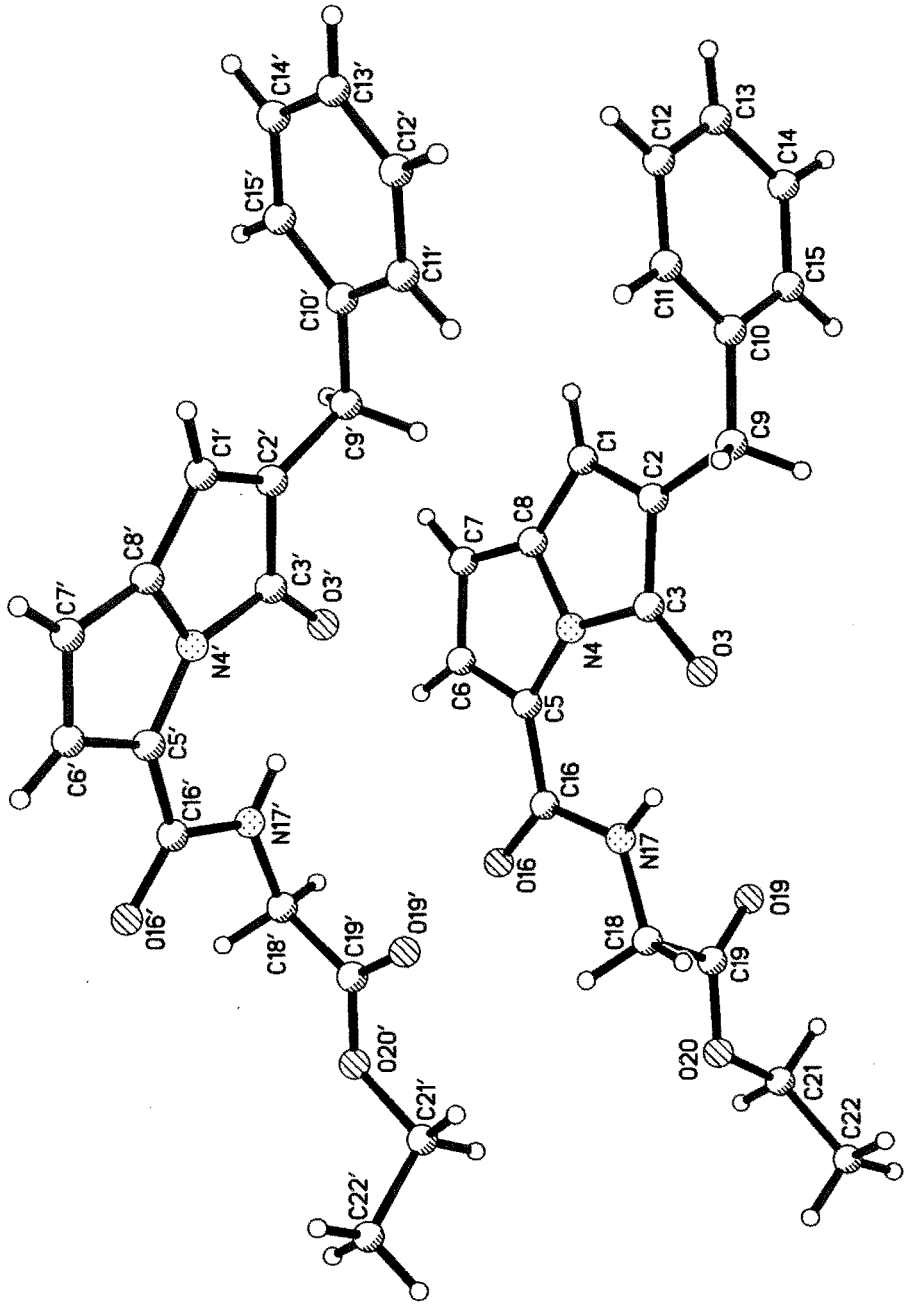
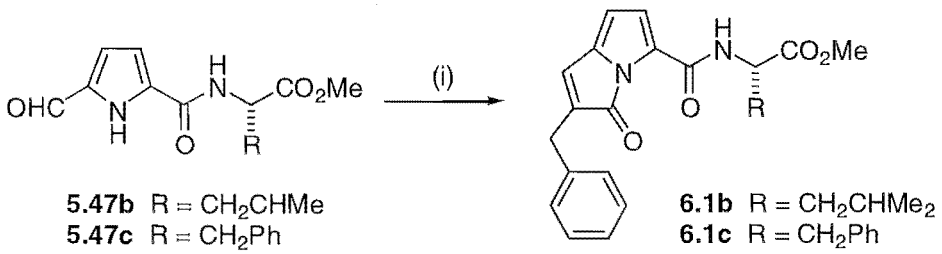



**Figure 6.2.** X-ray crystal structure of **6.1a** and **6.1a'** with crystallographic numbering scheme.

A search of the Cambridge Crystallographic Data Base revealed only one other X-ray crystal structure of a pyrrolizin-3-one, that of 6-bromopyrrolizin-3-one.[1] In agreement with the previously reported structure, our crystal structure of compound **6.1a** shows that the pyrrolizin-3-one ring is planar at all positions. Another notable similarity between the present crystal structure and the previously reported structure is that the C(3)-N(4) and C(3')-N(4') bond lengths of **6.1a** and **6.1a'** are significantly longer [1.406(3) and 1.408(3) Å, respectively] than that of a normal cyclic tertiary amide [1.335(9) Å].[2] These observations are consistent with reduced amide delocalisation in the pyrrolizin-3-one structure. This is further supported by the significant alternation of the bond lengths in the pyrrole ring, indicating a decrease in aromaticity relative to pyrrole.[3] Furthermore, the olefinic C(1)-C(2) and C(1')-C(2') bond lengths are relatively long [1.335(3) and 1.338(3) Å, respectively] which indicates there is only limited conjugation to the pyrrole ring.

Having unequivocally identified the product of the *N*-acylation reaction of **5.47a** as the pyrrolizin-3-one **6.1a**, further examples were prepared for comparison and to assess the scope of the reaction. Thus, reaction of **5.47b** and **5.47c** with hydrocinnamoyl chloride by the methodology described above gave the pyrrolizin-3-ones **6.1b** and **6.1c** in yields of 55% and 58%, respectively (Scheme 6.2). The structures of the compounds **6.1b** and **6.1c** were assigned by comparison of the $^1$H NMR spectral data with that of the previously determined pyrrolizin-3-one **6.1a**.




**5.47b** R = $CH_2CHMe$
**5.47c** R = $CH_2Ph$

**6.1b** R = $CH_2CHMe_2$
**6.1c** R = $CH_2Ph$

**Scheme 6.2.** *Reagents and conditions*: (i) DMAP, Hünigs base, $CH_2Cl_2$ then $Ph(CH_2)_2COCl$ (**6.1b**, 55%); (**6.1c**, 58%).

Characteristic $^1$H NMR and UV spectral data for the pyrrolizin-3-one derivatives **6.1a-c** are summarised in Tables 6.1 and 6.2.

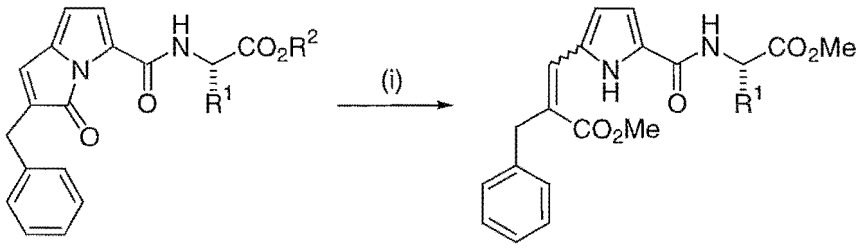
| Compound | H-1 | H-6 | H-7 | $CH_2Ph$ | NH |
|---|---|---|---|---|---|
| **6.1a** | 6.65, t (1.5) | 6.88, d (3.4) | 5.97, d (3.4) | 3.62, d (1.5) | 9.33, t (5.4) |
| **6.1b** | 6.61, t (1.7) | 6.87, d (3.4) | 5.96, d (3.4) | 3.62, d (1.7) | 9.22, d (6.8) |
| **6.1c** | 6.60, t (1.5) | 6.84, d (3.4) | 5.94, d (3.4) | 3.60, d (1.5) | 9.31, d (7.3) |

**Table 6.1.** Characteristic $^1$H NMR spectral data for pyrrolizin-3-ones **6.1a-c** [δ (300 MHz), $CDCl_3$, multiplicity (*J* coupling in Hz)].

| Compound | Solvent | $\lambda_{max}$ (ε) | | |
|---|---|---|---|---|
| **6.1a** | MeOH | 242 (11300) | 338 (25400) | 423 (200) |
| **6.1b** | MeOH | 223 (11500) | 339 (9100) | 436 (900) |
| **6.1c** | $CHCl_3$ | | 301 (12300) | 438 (4100) |

**Table 6.2.** UV spectra of pyrolizin-3-ones **6.1a-c** (nm).

During an attempt to purify the pyrrolizin-3-one **6.1b** from residual hydrocinnamic acid by eluting a small sample through an activated amino cartridge with methanol, evidence of a ring-opened product was observed. However, this compound was not characterised further. In light of this observation, separate samples of **6.1a-c** were treated using sodium methoxide in methanol to give colourless samples of the pyrrole acrylic esters **6.2a-c** in yields of 85%, 80% and 82%, respectively (Scheme 6.3).



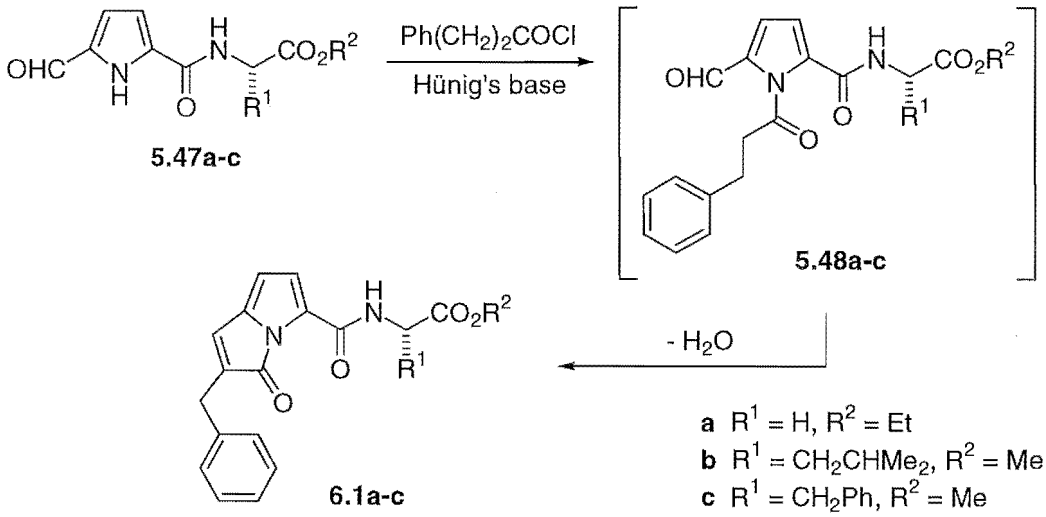

**6.1a** $R^1 = H$, $R^2 = Et$
**6.1b** $R^1 = CH_2CHMe_2$, $R^2 = Me$
**6.1c** $R^1 = CH_2Ph$, $R^2 = Me$

**6.2a** R = H
**6.2b** $R = CH_2CHMe_2$
**6.2c** $R = CH_2Ph$

**Scheme 6.3.** *Reagents and conditions*: (i) MeONa, MeOH (**6.2a**, 85%), (**6.2b**, 80%), (**6.2c**, 82%).

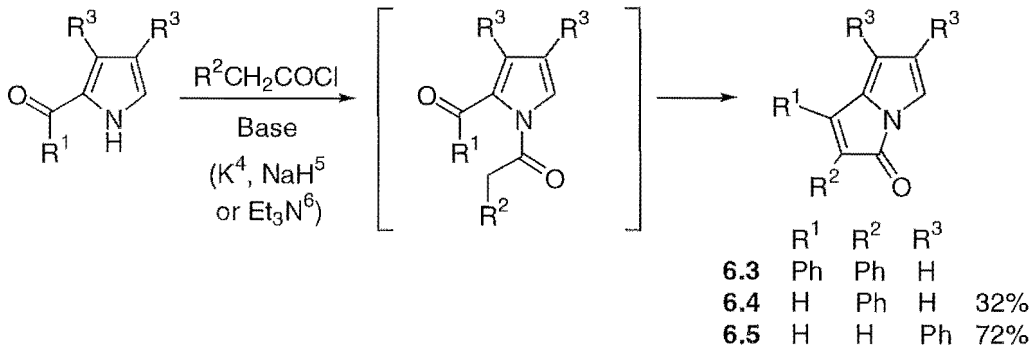
In the case of the leucine and phenylalanine examples **6.2b** and **6.2c**, only the *cis*-isomer was observed. However, in the case of the glycine example **6.2a**, a mixture of the *cis*- and *trans*-isomers were obtained in a ratio of 3 : 1 (by $^1$H NMR spectroscopy). Subsequent recrystallisation of this mixture gave a pure sample of the *trans*-isomer (22% yield), which was fully characterised. The assignment of a *cis*-configuration for the alkene of **6.2b** (and by analogy for **6.2a** and **6.2c**) was based on an observed strong positive NOE enhancement at δ 3.74 (*ie* C**H**$_2$Ph) upon irradiation at δ 6.65 (*ie* pyrr-C**H**). An NOE was also observed in the reverse direction on irradiation of C**H**$_2$Ph (δ 3.74).

A possible mechanism for the formation of compounds **6.1a-c** would involve initial *N*-acylation of the 5-formylpyrrole-2-carboxamides **5.47a-c**, followed by an intramolecular Knoevenagel type condensation catalysed by Hünig's base, to give the corresponding pyrrolizin-3-ones **6.1a-c** (Scheme 6.4). An interesting point to note in this mechanism is that the proposed *N*-acyl intermediates **5.48a-c** are in fact the compounds we were attempting to prepare (see Scheme 6.1).




**Scheme 6.4.** Proposed mechanism for the formation of the pyrrolizin-3-ones **6.1a-c**.

An examination of the literature revealed only three other reports of pyrrolizin-3-ones having been prepared through an analogous intramolecular Knoevenagel type condensation of an *N*-acylated-2-acylpyrrole intermediate.[4-6] The products in these reactions were identified as the pyrrolizin-3-ones **6.3**,[4] **6.4**[5] and **6.5**[6] (Scheme 6.5).

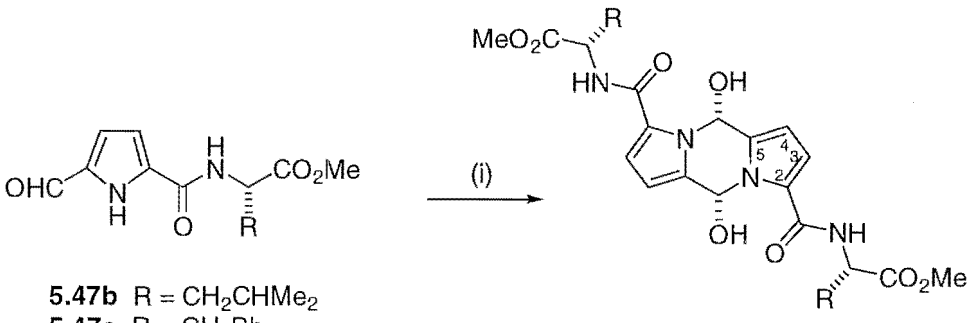



**Scheme 6.5.** Other pyrrolizin-3-ones formed via a Knoevenagel type condensation.

The more common approach to synthesise pyrrolizin-3-ones is to form the 3,4-lactam bond as the final step in ring formation, through the use of an intramolecular acylation of the pyrrole nitrogen. To enable this intramolecular *N*-acylation step, however, a suitably activated acyl substituent is required at the pyrrole 2-position. The main methodologies by which this acyl group has been activated, and hence formation of a pyrrolizin-3-one achieved, include formation of a mixed anhydride followed by ring closure and decarboxylation,[5] through a base catalysed elimination from pyrrolepropenoic and propanoic derivatives,[7] and by flash vacuum pyrolysis (FVP) of a Meldrum's acid or propenoate ester derivative of pyrrole.[8] A number of other methodologies for the formation of pyrrolizin-3-ones have also been reported in the literature.[9] However, these methodologies require the concerted formation of two bonds to effect ring formation, either through the use of a ketene intermediate[10] or as a result of a thermal elimination after a Vilsmeier-Haack acylation reaction of pyrroles with certain amides.[11]

## 6.2. Attempted N-acylation of 5.47 using sodium hydride methodology: Azafulvene dimer formation

An attempt was then made to *N*-acylate the 5-formylpyrrole-2-carboxamides **5.47** using the sodium hydride methodology described in Chapter 2. In separate experiments, a sample of each of **5.47b** and **5.47c** were treated with an equivalent of sodium hydride, and the resulting pyrrolyl anion was reacted with an equivalent of acetyl chloride. Work up of the reaction mixture and purification of the resulting residue by flash chromatography on silica gave the azafulvene dimers **6.6b** and **6.6c** in yields of 41% and 25%, respectively (Scheme 6.6). Polymeric material was also obtained from this reaction sequence. Significantly, the dimers **6.6b** and **6.6c** were subsequently found, by X-ray crystallographic analysis and observed optical rotation, to be present as only a single stereoisomer, which had a *syn*-diol relative configuration (see later for a discussion). No evidence of any of the other possible isomers were observed in the $^1$H NMR spectra of the crude products from these reactions. When the above reaction of **5.47b** was repeated using hydrocinnamoyl chloride in place of acetyl chloride a reduced yield of **6.6b** was obtained (25%). This was largely due to difficulties in purifying the desired product **6.6b** from residual hydrocinnamic acid.




**5.47b** R = $CH_2CHMe_2$
**5.47c** R = $CH_2Ph$

**6.6b** R = $CH_2CHMe_2$
**6.6c** R = $CH_2Ph$

**Scheme 6.6.** *Reagents and conditions*: (i) NaH, THF then either $CH_3COCl$ (**6.6b**, 41%), (**6.6c**, 25%) or $Ph(CH_2)_2COCl$ (**6.6b**, 25%).

The dimeric nature of **6.6b** and **6.6c** were proposed on the basis of high resolution mass spectra which were consistent with the structural formulae $C_{26}H_{35}N_4O_7$ (MH-$H_2O$) and $C_{32}H_{31}N_4O_7$ (MH-$H_2O$), respectively. The proposed structures of **6.6b** and **6.6c** were further supported by a series of one and two dimensional NMR experiments. Key HMBC correlations for **6.6c** were found between the proton resonance at δ 6.23 (*ie* pyrrole-**C**H**O**H-N) and the carbon resonance at δ 130.7 (*ie* pyrrole **C**5, see Scheme 6.6 for pyrrole numbering), and between the proton resonance at δ 6.17 (*ie* pyrrole **H**4) and the carbon resonances at δ 130.7 and 71.5 (*ie* pyrrole **C**5 and pyrrole-**C**H**O**H-N, respectively). These key HMBC correlations supported our proposed dimeric structure. Furthermore, the optical rotary dispersion measurements in dichloromethane of $[\alpha]_D^{25}$ – 120.6° and –149.0° for **6.6b** and **6.6c**, respectively, indicated that they were optically active species.

In order to confirm our structural assignment for **6.6b** and **6.6c**, and also to determine both the relative configuration of the diols (*ie syn* or *anti*) and the absolute configuration of the dimers, we set about growing crystals of **6.6b** and **6.6c** suitable for X-ray crystallographic analysis. After several initial attempts, suitable crystals of **6.6b** and **6.6c** were eventually obtained, from the solvent system ethyl acetate/petroleum ether, by employing the crystallisation technique of vapour diffusion.

The single crystal X-ray analysis of **6.6b** showed that the molecule crystallised in the chiral space group $P4_22_12$. The asymmetric unit was also shown to contain a disordered solvent molecule of ethyl acetate in addition to the **6.6b** molecule. A perspective drawing of **6.6b**, with atomic labelling, is presented in Figure 6.3a. Two molecules pack (*ie* dimerise) such that they are related by a 2-fold rotational axis, having their central rings cofacial and the pyrrole rings offset by 45° (see Figures 6.3b and 6.3c). The distance between the planes of these molecules ranges from 3.5 – 4.0 Å. This arrangement is similar to π-π stacking as reported for related porphyrin systems in which two porphyrin molecules adopt a cofacial arrangement with their centres offset, to give an interplanar separation of 3.4 – 3.6 Å.[12] Another key point to note in the crystal structure

of **6.6b** is that hydrogen bonds are also evident between the amide hydrogens of one molecule and the ester carbonyl groups of the other (*ie* O9' to N7 distance is 2.965 Å).

(a)

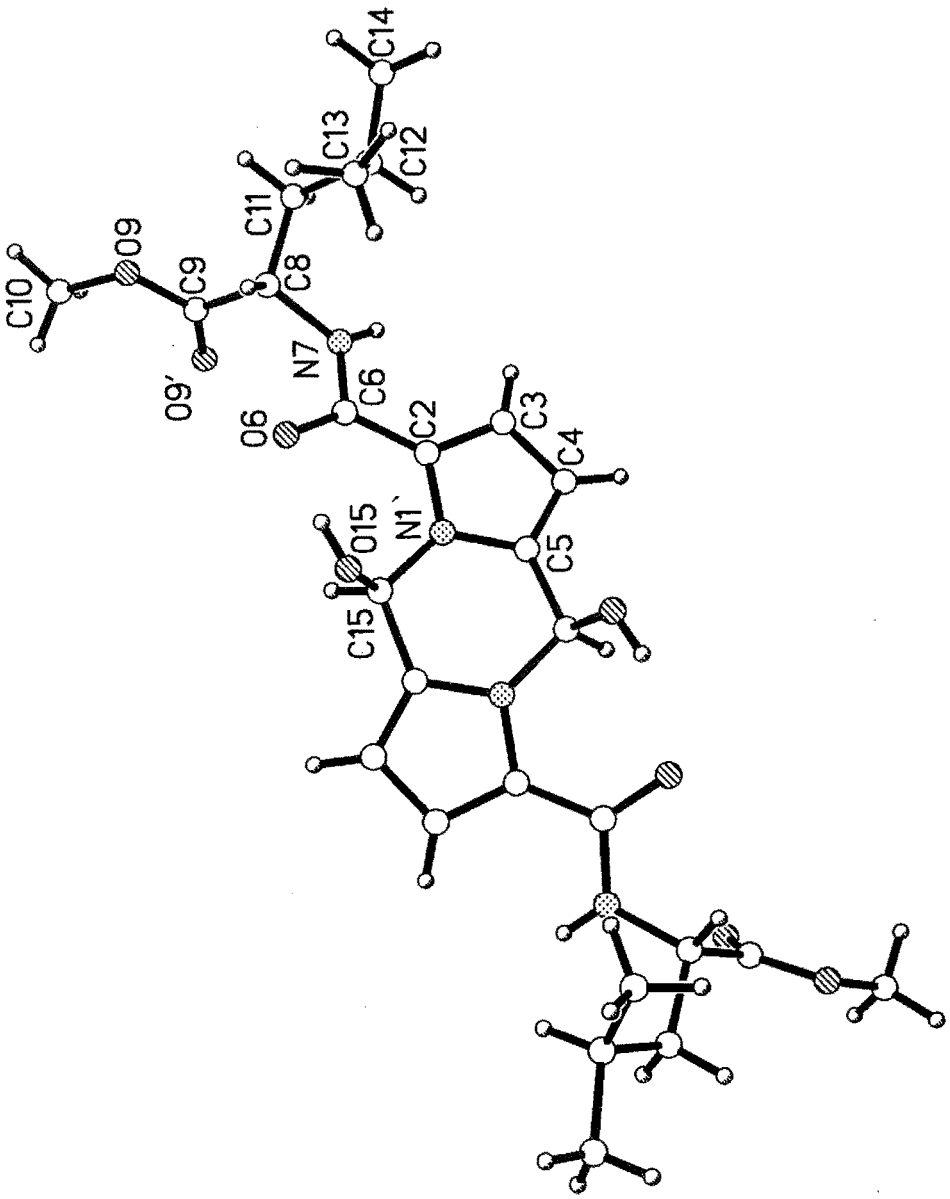
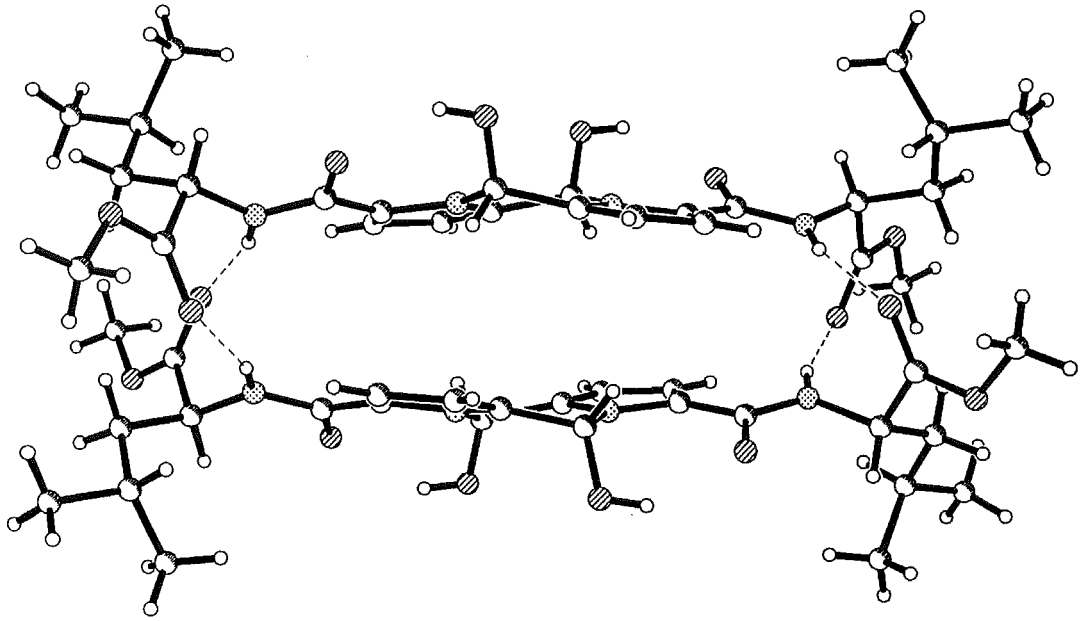
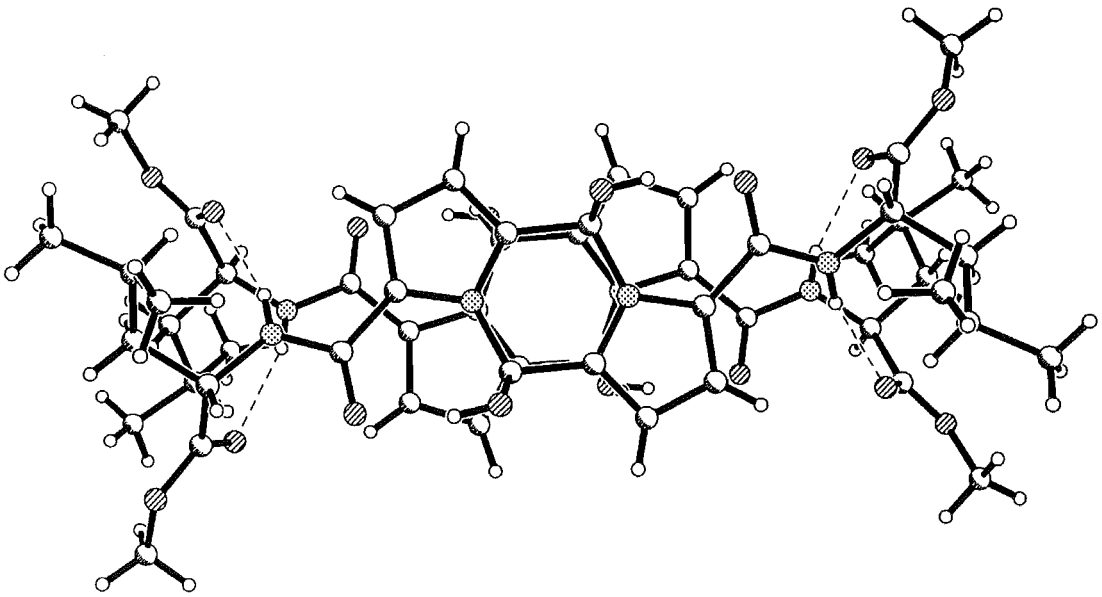



**Figure 6.3.** (a) X-ray crystal structure of compound **6.6b** with crystallographic numbering scheme (for clarity the co-crystallised ethyl acetate molecule is not shown). (b) Side view showing non-covalent dimerisation. (c) Top view showing overlay of pyrrole rings in the non-covalent dimer.

(b)



(c)



A single crystal X-ray analysis of the phenylalanine-based example **6.6c** also revealed the same non-covalent dimerisation as seen in **6.6b** (see Figures 6.4a-c for perspective drawings of **6.6c**). The important points to note from the X-ray crystal structures of **6.6b** and **6.6c**, therefore, are that the dimers **6.6b** and **6.6c** are present as single diastereomers which have only one of the two possible *syn*-configurations of the diols, and that these *syn*-diol compounds are further dimerised by non-covalent interactions.

(a)

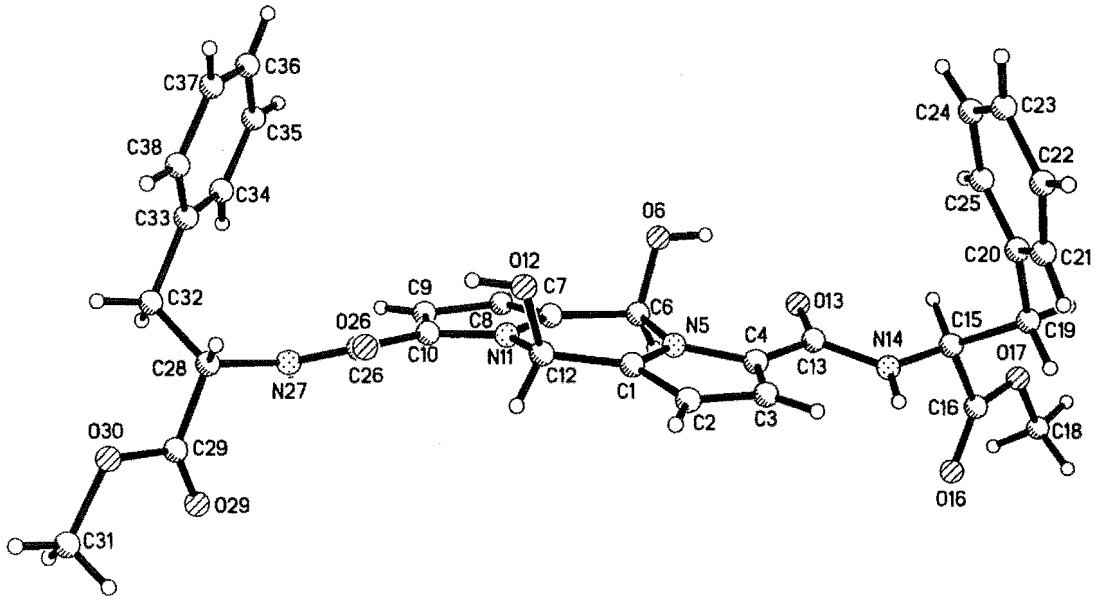
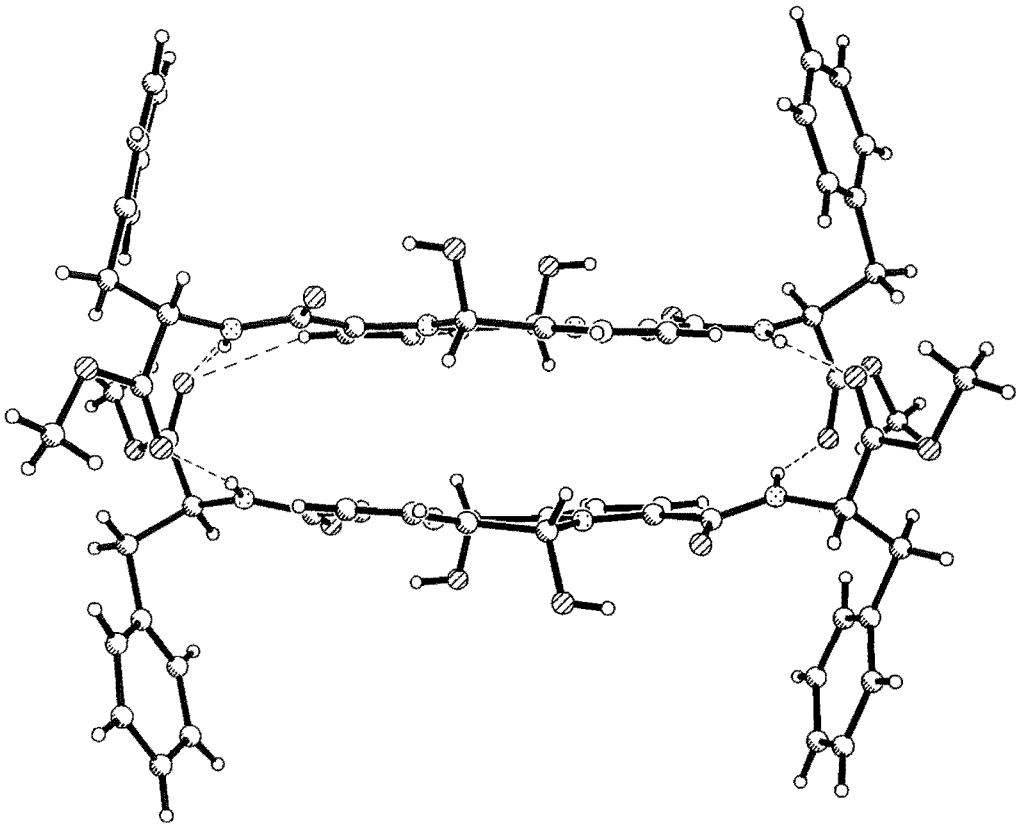
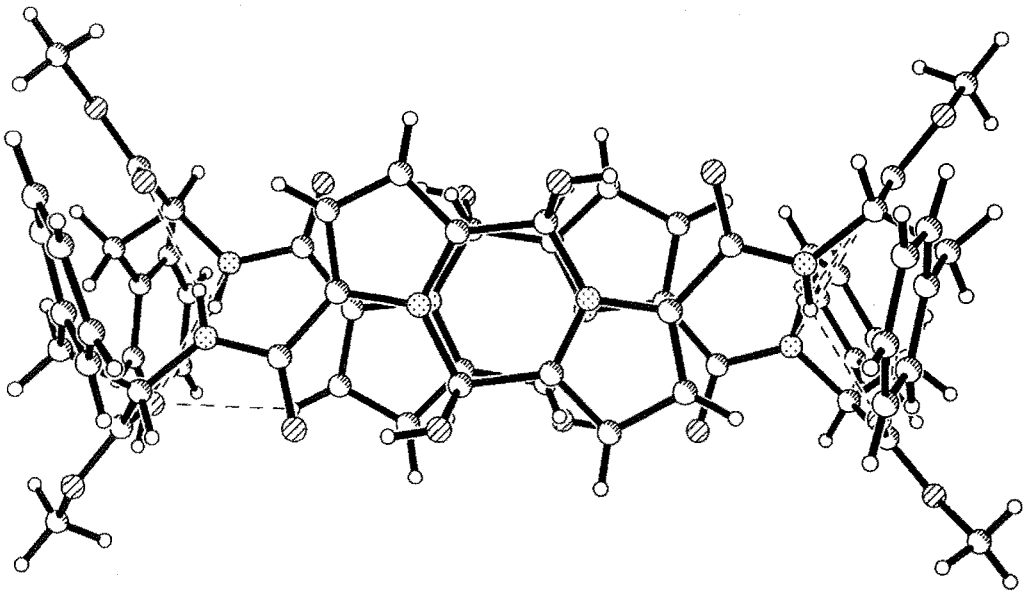



**Figure 6.4.** (a) X-ray crystal structure of compound **6.6c** with crystallographic numbering scheme. (b) Side view showing non-covalent dimerisation. (c) Top view showing overlay of pyrrole rings in the non-covalent dimer.

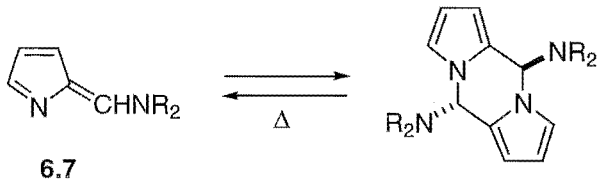
(b)



(c)



Another important point to note is that 6-amino-1-azafulvenes of type **6.7** are known to dimerise to give the analogous *anti*-dipyrrolo[1,2-a:1',2'-d]pyrazines **6.8** (Scheme 6.7).[13] The formation of the dimer **6.8** is reversible, and the 6-amino-1-azafulvene **6.7** can be regenerated by a thermal decomposition of the dimer.[14] The *anti*-configuration of compounds of type **6.8** has been assigned on the basis of $^1$H NMR spectroscopy[14] and X-ray crystallography.[15] This preference for the *anti*-isomer of **6.8** contrasts with our examples (*ie* **6.6b** and **6.6c**) where the *syn*-isomers were produced exclusively as a single diastereomer under mild conditions.




**6.7** **6.8**

**Scheme 6.7.** Dimerisation/thermal decomposition of 6-amino-1-azafulvenes.

As only a single diastereomer of **6.6b** and **6.6c** were produced in the reaction sequence described above, we postulate that the diastereoselective synthesis of dimers **6.6b** and **6.6c** from the corresponding pyrrole carboxamides **5.47b** and **5.47c** was controlled by their ability to form the non-covalent dimeric structures in solution, *ie* this represents an example of molecular self-assembly. Molecular self-assembly has been defined by Whitesides *et al.*[16] as "the spontaneous association of molecules under equilibrium conditions into stable, structurally well-defined aggregates joined by non-covalent bonds." As such, the self-assembly of molecules to form well-defined supramolecular structures under the influence of weak, non-covalent forces such as hydrogen bonds, electrostatic interactions and hydrophobic interactions, is an important area of current chemical and biochemical research.[16-20] Numerous examples of self-assembly can be found in nature, such as in the spontaneous formation of the double helix

of nucleic acids, in the stacking of the viral protein coat of the tobacco mosaic virus (TMV), and in the formation of multiprotein complexes.[17] Furthermore, self-assembly also has potential in the development of strategies for the preparation of non-biological nanostructures.[16]

In related peptidic examples, cyclic peptides such as the eight-residue *cyclo*[(–*L*-Phe-*D*-*N*-MeAla–)$_4$] **6.9**, which have a selectively *N*-methylated backbone, have been shown to self-assemble into discrete, cylindrical dimers of the type **6.10** (Figure 6.5).

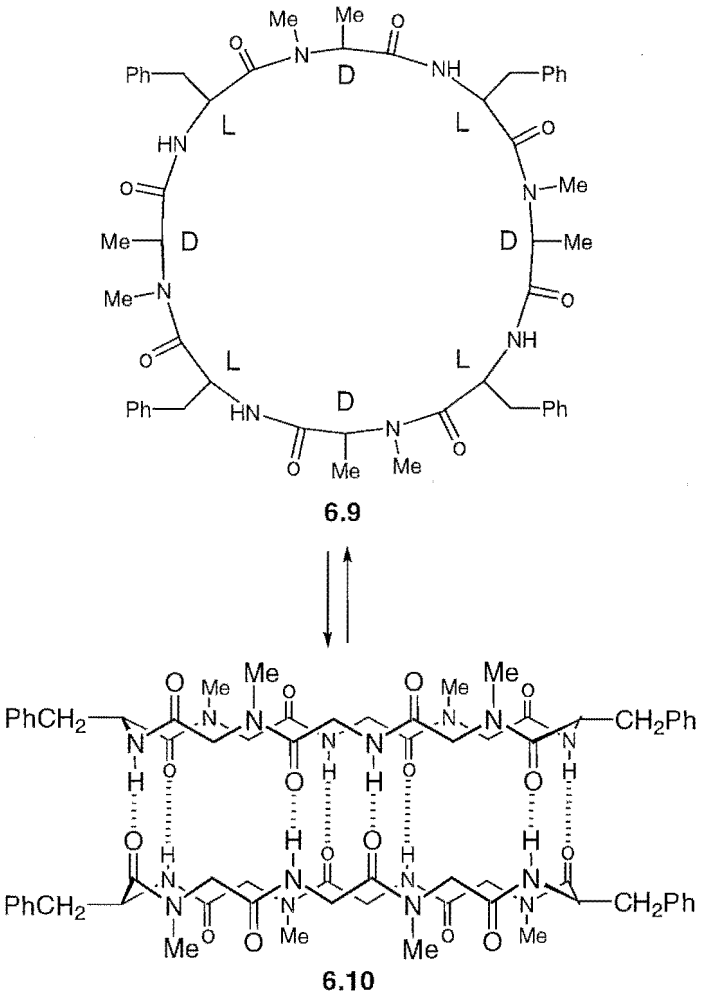



**Figure 6.5.** A two dimensional representation of the chemical structure of the peptide subunit **6.9** (*L* and *D* refers to the chirality of the amino acid) and the self-assembled cylindrical dimer **6.10** (for clarity only four of the eight $CH_2Ph$ groups are represented).

In these examples, the backbone *N*-methyl groups block one face of the flat cycle to further hydrogen bonding.[18] In the absence of such *N*-methylation, however, cyclic peptides such as **6.9** self-assemble, by extended hydrogen bonding, into open-ended, hollow, tubular structures that act as simple models of ion channelling systems.[18] The introduction of aromatic rings into the cycles has also been shown to facilitate self-assembly due to π-π stacking, rather than hydrogen bonding.[19] Our current examples **6.6b** and **6.6c** are analogous to the first of these literature-based systems, where the *syn*-hydroxyl groups block one face of the dimer molecule such that non-covalent interactions on the other face of the molecule then result in the formation of the non-covalent dimers shown in Figures 6.3 and 6.4. However, an important difference in our examples **6.6b** and **6.6c** compared to the previous literature-based systems, is that the non-covalent association is thought to control the diastereoselective synthesis of these compounds. A final important point to note is that the alternative *syn*- and *anti*-diol configurations for **6.6b** and **6.6c** would block the formation of the non-covalent dimeric structures of these compounds due to steric reasons. This may account for why only one of the two possible *syn*-diol configurations was observed to form in the above reaction.

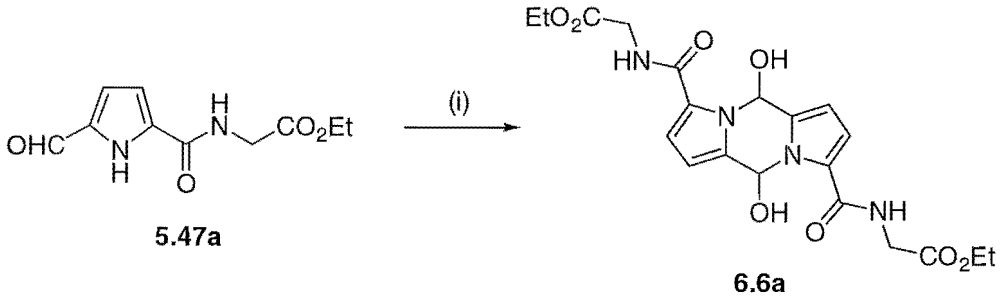
For the synthesis of the dimers **6.6b** and **6.6c** to be a molecular self-assembly process, however, requires the non-covalent dimeric structures of **6.6b** and **6.6c** to be formed under equilibrium conditions (*ie* in solution) during the reaction sequence. Evidence which suggests that these non-covalent dimers of **6.6b** and **6.6c** are indeed present in solution was obtained by examining both the frequency of the amide N-H stretches in the infrared spectra and the chemical shifts of the N-H protons in the $^1$H NMR spectra. The appearance of an N-H stretching band at 3342 and 3352 cm$^{-1}$ in the chloroform infrared spectra of **6.6b** and **6.6c**, respectively, was consistent with hydrogen bonding in solution as reported for the peptide dimer **6.10** (Figure 6.5).[18a] In this previous example, the formation of a tight hydrogen-bonded dimer, with an average intersubunit N-O distance of 2.95 Å, was shown by the appearance of an N-H stretching band at 3309 cm$^{-1}$ in the infrared spectrum.[18a] In the case of our example **6.6b**, the X-ray

crystal structure had shown a similar tight hydrogen-bonded dimer, with an intersubunit N-O distance of 2.965 Å (see Figure 6.3b and earlier for a discussion). In addition, the significant downfield shifts (~1.2 – 1.4 ppm) of the amide proton resonances in $CDCl_3$ upon going from the starting pyrrole carboxamides **5.47b** and **5.47c** to the azafulvene dimers **6.6b** and **6.6c** (*ie* δ 7.03 to 8.46 and δ 6.99 to 8.16, respectively) was also consistent with the occurrence of this hydrogen bonding in solution.[18a,19,20] In agreement, the cyclic peptide subunit **6.9** (Figure 6.5) also showed a significant downfield shift of 1.75 ppm for the phenylalanine N-H resonance in the $^1$H NMR spectrum upon formation of the self-assembled peptide dimer **6.10** (*ie* δ 6.98 to 8.73).[18a]

To further determine whether the non-covalent dimers of **6.6b** and **6.6c** were present in solution, $^1$H NMR spectra of **6.6b** and **6.6c** were obtained in the more polar solvents DMSO-$d_6$ and acetone-$d_6$ (and also in $CD_3OD$ for **6.6b**). In such polar solvents it might be expected that the solvent would be able to disrupt the hydrogen bonds between the non-covalent dimers of **6.6b** and **6.6c**, and so effect disaggregation of the individual dimers. Any such non-covalent dimer disaggregation would then be shown in the $^1$H NMR spectra by the appearance of new upfield proton resonances corresponding to the (solvated) amide groups of the individual dimers. However, the $^1$H NMR spectra of **6.6b** and **6.6c** in these more polar solvents failed to show any evidence of new resonances for the amide protons. Consequently, the above $^1$H NMR spectral results either indicate that no such intermolecular hydrogen bonding between the individual dimer molecules of **6.6b** and **6.6c** was occurring in solution, or conversely, that the non-covalent interactions between the dimers was so strong that the solvent was unable to disrupt these interactions and effect disaggregation. Further work is therefore required in order to establish whether or not such non-covalent dimerisation of **6.6b** and **6.6c** is in fact occurring in solution, and hence directing the diastereoselective synthesis of these dimers.

Next, we set out to investigate whether the non-chiral glycine-based pyrrole carboxamide **5.47a** would also give the corresponding azafulvene dimer **6.6a** as a single

stereoisomer during the above reaction sequence. Consequently, a sample of **5.47a** was treated with an equivalent of sodium hydride, followed by the addition of an equivalent of acetyl chloride. A $^1$H NMR spectrum of the crude product from this reaction indicated the presence of a single isomer of **6.6a**. Purification of this product by flash chromatography on silica gave the azafulvene dimer **6.6a** in a yield of 50% (Scheme 6.8).




**5.47a** **6.6a**

**Scheme 6.8.** *Reagents and conditions*: (i) NaH, THF then $CH_3COCl$ (50%).

The $^1$H NMR spectrum of the purified dimer **6.6a** once again indicated only a single isomer. However, due to the presence of symmetry within the molecule, we were unable to ascertain by $^1$H NMR spectroscopy whether the dimer **6.6a** had a *syn-* or *anti-*configuration of the diols. On the basis of our previous dimer examples **6.6b** and **6.6c**, we would expect **6.6a** to have an analogous *syn*-configuration of the diols, and also to be further dimerised by non-covalent interactions such as hydrogen bonding. Evidence which indicated that this may indeed be the case for the dimer **6.6a** was shown in the infrared and $^1$H NMR spectra. The appearance of an N-H stretching band at 3350 cm$^{-1}$ in the chloroform infrared spectrum of **6.6a**, and a large downfield shift of 1.58 ppm for the amide proton resonance upon going from **5.47a** to **6.6a** (*ie* δ 6.94 to 8.52), were consistent with our previous examples **6.6b** and **6.6c**, and therefore imply hydrogen bonding in solution (see earlier for a discussion). This would indicate, therefore, that the glycine-based azafulvene dimer **6.6a** also had a *syn*-configuration of the diols. However, with such an arrangement the dimer **6.6a** would be expected to be racemic, since both

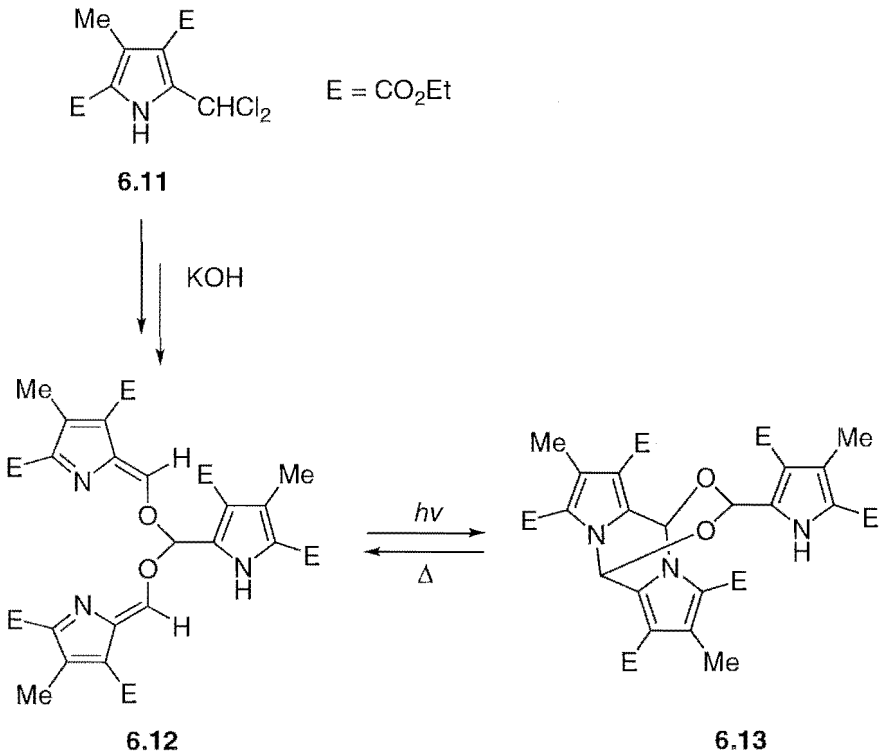
*syn*-diol configurations of **6.6a** would allow the non-covalent interactions discussed above.

In an effort to determine unequivocally the relative configuration of the diols in the glycine dimer **6.6a**, several attempts were made to grow crystals of **6.6a** suitable for X-ray crystallographic analysis. However, despite numerous crystallisation attempts we were unable to obtain crystals suitable for X-ray analysis. Consequently, the definitive answer as to the configuration of the diols of **6.6a** remains unresolved, although as discussed above, a *syn*-configuration would seem most likely. Another important point to note regarding **6.6a** was that on standing for 3 days in $CDCl_3$, approximately 15% of another compound, tentatively assigned as the *anti*-isomer, was observed to form. In contrast, compounds **6.6b** and **6.6c** were stable for extended periods in $CDCl_3$, with no sign of any such epimerisation.

The mechanism for the formation of the azafulvene dimers **6.6a-c** is undoubtedly complex, especially in the case of **6.6b** and **6.6c** where we found only a single stereoisomer was produced. The exact role of the acid chloride in the formation of the dimers **6.6** remains unclear, except to say that the reactions do not proceed in its absence. It is possible that the acid chloride reacts with the pyrrolyl anion, generated on reaction of **5.47** with sodium hydride, through the oxygen of the formyl group, in an analogous procedure to that described in the formation of the 6-acetyloxy-1-azafulvene dimer **5.38** (see Scheme 5.17, Chapter 5). The resulting *O*-acylazafulvene intermediate could then react with another pyrrolyl anion to give **6.6** after hydrolysis. However, at this moment there is no direct evidence for this *O*-acylazafulvene intermediate since no *O*-acylated side products have been isolated from the above reactions. Consequently, further work is required in this area in order to establish the exact mechanisms involved in the formation of the azafulvene dimers **6.6**.

Finally, in a possibly related reaction, Brittain *et al.*[21] in 1981 reported that diethyl 2-dichloromethyl-4-methylpyrrole-3,5-dicarboxylate **6.11** reacted specifically with aqueous potassium hydroxide to yield the thermochromic tripyrrolic system **6.13**

(Scheme 6.9). The structure of **6.13** was unequivocally established by way of X-ray crystallographic analysis. The mechanism of the formation of **6.13** was not totally understood, although it has since been suggested that the potassium ion acts as a template ion for the initial formation of **6.12**.[22] The important point to note from this earlier example, however, was that the *syn*-isomer was formed exclusively from the above hydrolysis reaction. Here, a covalent linkage, rather than a non-covalent association as postulated for the azafulvene dimers **6.6**, determined the stereochemical outcome of the reaction.




**Scheme 6.9.** Formation of the thermochromic tripyrrolic system **6.13** (Brittain *et al.*).

## 6.3. Summary

During attempts to *N*-acylate the 5-formylpyrrole-2-carboxamides **5.47a-c** using standard acylation methodologies, we have observed the formation of unexpected heterocyclic side products.

When the *N*-acylation reaction was attempted using hydrocinnamoyl chloride and the DMAP methodology, a series of orange/red compounds were isolated. These compounds were identified as the pyrrolizin-3-ones **6.1a-c** on the basis of one and two dimensional NMR spectral techniques. Subsequent conformation of the structure of the pyrrolizin-3-one **6.1a** was achieved by a single crystal X-ray analysis. The most likely mechanism for pyrrolizin-3-one formation is the initial *N*-acylation of the formylpyrrole followed by an intramolecular Knoevenagel type condensation.

When the *N*-acylation reaction was attempted using an acid chloride and the sodium hydride methodology, the azafulvene dimers **6.6a-c** were obtained. These compounds were identified on the basis of one and two dimensional NMR spectral techniques. Subsequent single crystal X-ray analysis of the leucine and phenylalanine-derived dimers **6.6b** and **6.6c** revealed that each of these compounds were present as only a single diastereomer, and were further dimerised by non-covalent interactions such as hydrogen bonding. Consequently, we have suggested that the diastereoselective synthesis of the azafulvene dimers **6.6b** and **6.6c** is controlled by this ability to form the non-covalent dimeric structures in solution, and as such, represents a possible example of molecular self-assembly. However, further work is required to more fully understand these observations.

## 6.4. References for chapter six


1 Blake, A. J.; McNab, H.; Morrison, R. *J. Chem. Soc., Perkin Trans. I.* **1988**, 2145.

2 Chakrabarti, P.; Dunitz, J. D. *Helv. Chim. Acta.* **1982**, *65*, 1555.

3 Nygaard, L.; Nielsen, J. T.; Kirchheiner, J.; Maltesen, G.; Rastrup-Andersen, J.; Sørensen, G. O. *J. Mol. Struct.* **1969**, *3*, 491.

4 Carelli, V.; Cardellini, M.; Morlacchi, F. *Ann. Chim. (Rome)* **1961**, *51*, 595.

5 Flitsch, W.; Neumann, U. *Chem. Ber.* **1971**, *104*, 2170.

6 Dannhardt, G.; Steindl, L. *Arch. Pharm. (Weinheim, Ger.)* **1986**, *319*, 745.

7 For an example see: Filippovich, E. I.; Evstigneeva, R. P.; Preobrazhenskii, N. A. *Zh. Obshch. Khim. (Engl. Transl.)* **1960**, *30*, 3223.

8 For recent examples see: (a) Campbell, S. E.; Comer, M. C.; Derbyshire, P. A.; Despinoy, X. L. M.; McNab, H.; Morrison, R.; Sommerville, C. C.; Thornley, C. *J. Chem. Soc., Perkin Trans. I.* **1997**, 2195; (b) McNab, H.; Thornley, C. *J. Chem. Soc., Perkin Trans. I.* **1997**, 2203; (c) Comer, M. C.; Despinoy, X. L. M.; Gould, R. O.; McNab, H.; Parsons, S. *J. Chem. Soc., Chem. Commun.* **1996**, 1083.

9 For a recent review see: McNab, H.; Thornley, C. *Hetereocycles* **1994**, *37*, 1977.

10 For an example see: Bestmann, H, J,; Bansal, R. K. *Tetrahedron Lett.* **1981**, *22*, 3839.

11 For an example see: Ermili, A.; Castro, A. J.; Westfall, P. A. *J. Org. Chem.* **1965**, *30*, 339.

12 (a) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525; (b) Hunter, C. A. *Chem. Soc. Rev.* **1994**, 101.

13 Kobayashi, T.; Kajigaeshi, S.; Kanemasa, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3255.

14 Mori, S.; Watanabe, M.; Kajigaeshi, S.; Kanemasa, S. *Heterocycles* **1976**, *4*, 957.

[15] Veith, M.; Zimmer, M.; Huch, V. *Organometallics* **1993**, *12*, 1012.

[16] Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312.

[17] Lehn, J. –M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304, and references therein.

[18] (a) Ghadiri, M. R.; Kobayashi, K.; Granja, J. R.; Chadha, R. K.; McRee, D. E. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 93; (b) Hartgerink, J. D.; Clark, T. D.; Ghadiri, M. R. *Chem. Eur. J.* **1998**, *4*, 1367, and references therein.

[19] Ranganathan, D.; Haridas, V.; Gilardi, R.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 10793.

[20] (a) Clark, T. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, *117*, 12364; (b) Fan, E.; van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369; (c) Kobayashi, K.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 95.

[21] Brittain, J. M.; Jones, R. A.; Jones, R. O.; King, T. J. *J. Chem. Soc., Perkin Trans. I.* **1981**, 2656.

[22] Jones, R. A. *Comprehensive Heterocyclic Chemistry.* Katritzky, A. R.; Rees, C. W. Eds. Pergamon Press: Oxford, 1984, Vol 4, pp274.

# CHAPTER SEVEN

# SYNTHESIS AND BIOACTIVITY OF HYDROXYMETHYLPYRROLE-BASED METABOLITES

## 7.1. Introduction

Marine organisms, such as sponges, bryozoans and molluscs, have long been known as sources of novel compounds which possess potential pharmaceutical properties and/or synthetically interesting structures.[1] The different classes of compounds that have been characterised from these marine sources include terpenes, lipids, polyketides, peptides and amino acid-derived compounds, and other nitrogenous compounds. This last class of compounds can be further divided up into different subclasses depending on the nitrogenous heterocycle that the natural product is based upon. For example, a number of compounds based on the heterocycles indole, carbazole, pyridine and pyrrole have been described, along with a number of guanidine-containing and nucleoside-based compounds (see Figure 7.1 for representative examples).[1]

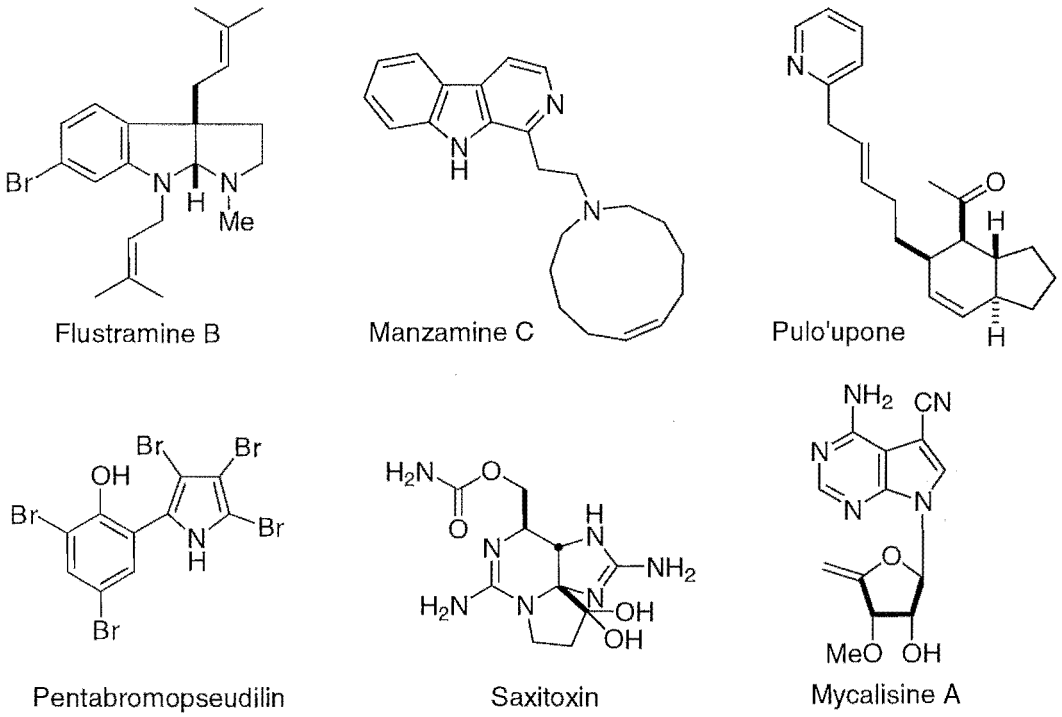



**Figure 7.1.** Representative examples of nitrogen heterocycle-based natural products.

In 1997, Ortega *et al.* isolated a series of fourteen novel pyrrole-based metabolites from a north-eastern Atlantic sponge *Mycale micracanthoxea*.[2] This work reported the isolation and characterisation of twelve 5-acyl-2-hydroxymethylpyrroles, named

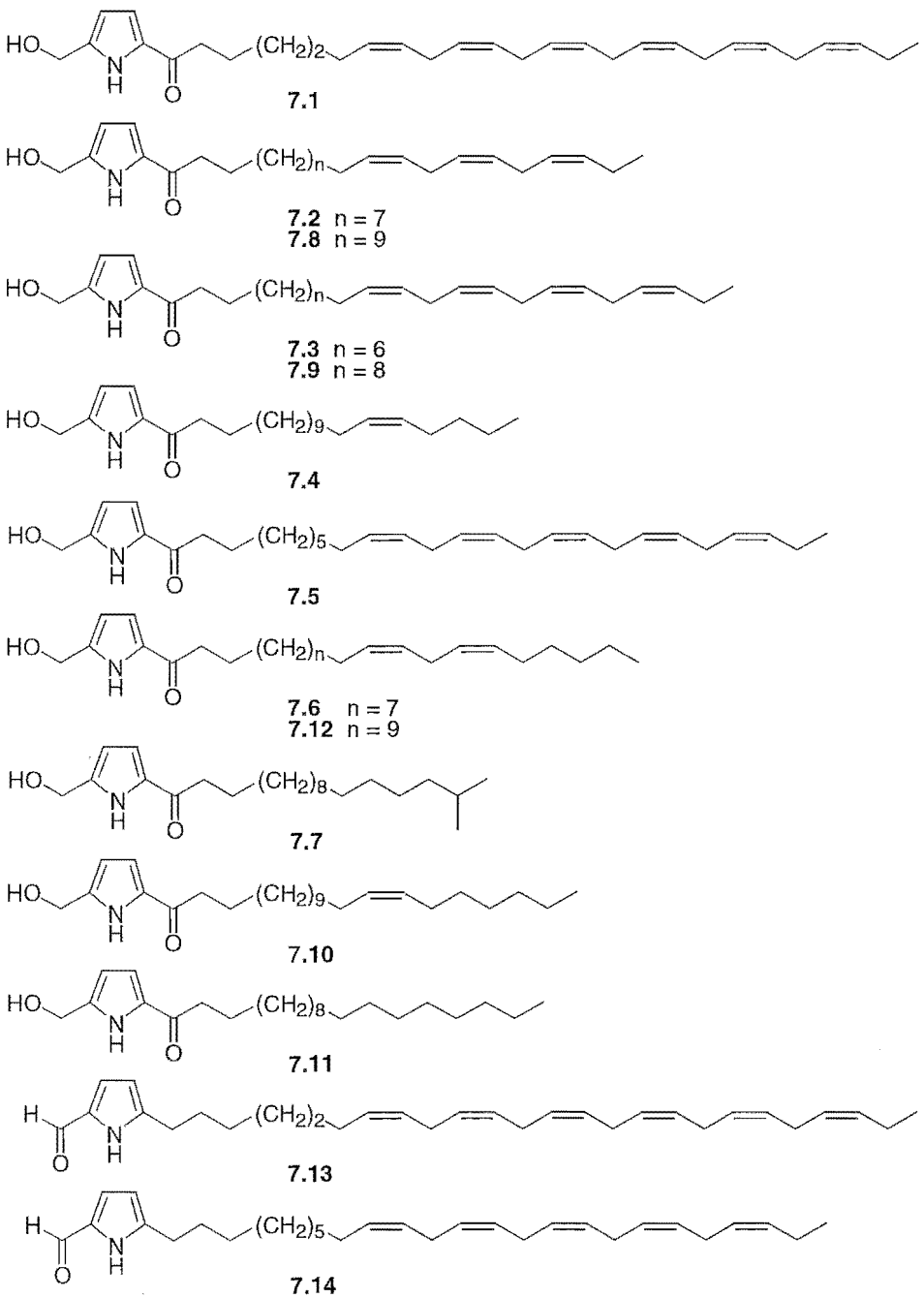


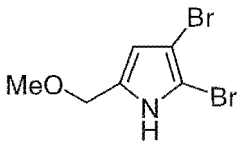

**Figure 7.2.** Mycalazols 1 – 12 (**7.1 – 7.12**) and mycalazals 1 – 2 (**7.13 – 7.14**).

mycalazols 1 – 12 (**7.1 – 7.12**), along with two 5-alkylpyrrole-2-carboxaldehydes, named mycalazals 1 – 2 (**7.13 – 7.14**), that differ in the length and degree of unsaturation of their side chain (Figure 7.2). These novel pyrrole-based metabolites were reported to show moderate cytoxicity ($ED_{50}$ of 1 – 10 μg $mL^{-1}$) against the P388 murine leukemia, SCHABEL mice lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma and MEL28 human melanoma cell lines (see Table 7.1).

| Assay | 7.1 | 7.2 | 7.3 | 7.4 | 7.5 | 7.6 | 7.7 | 7.8 | 7.9 | 7.10 | 7.11 | 7.12 | 7.14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P388 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2.5 | 1 | 2 |
| SCHABEL | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2.5 | 2 | 2 |
| A549 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 5 |
| HT29 | 2 | 5 | 2.5 | 2.5 | 2.5 | 1 | 2 | 5 | 2 | 2.5 | 10 | 10 | 5 |
| MEL28 | 2.5 | 5 | 2.5 | 2.5 | 2.5 | 2 | 2 | 2.5 | 2 | 2.5 | 10 | 5 | 5 |

**Table 7.1.** Cytotoxicity data ($ED_{50}$, μg $mL^{-1}$) of the mycalazols 1 – 12 (**7.1 – 7.12**) and mycalazal 2 **7.14**.

The mycalazols 1 – 12 were the first reported examples of 5-acyl-2-hydroxymethylpyrrole-based natural products, although the related methoxymethylpyrrole **7.15** had previously been reported by Tada and Tozyo as an extremely unstable metabolite from the marine sponge *Agelas sp.*[3]




**7.15** 2,3-dibromo-5-methoxymethylpyrrole

An examination of the literature revealed that a number of 5-alkylpyrrole-2-carboxaldehydes related to the mycalazals 1 – 2 had previously been isolated from marine sources. In 1980, Stierle and Faulkner reported the isolation of four 5-

alkylpyrrole-2-carboxaldehydes **7.16** differing in alkyl chain length, along with the pyrrole nitrile **7.17** and cyanohydrin **7.18**, from the marine sponge *Laxosuberites sp.* (Figure 7.3).[4] In 1984, Bowden *et al.* isolated 5-nonylpyrrole-2-carboxaldehyde **7.19** from a soft coral-sponge association of the genus *Telesto*.[5] A series of saturated, mono- and di-unsaturated 3-alkylpyrrole-2-carboxaldehydes **7.20 – 7.23** have been reported by Cimino *et al.* from the marine sponge *Oscarella lobularis*,[6] although it has since been suggested that they might also be 2,5-disubstituted pyrroles.[4]

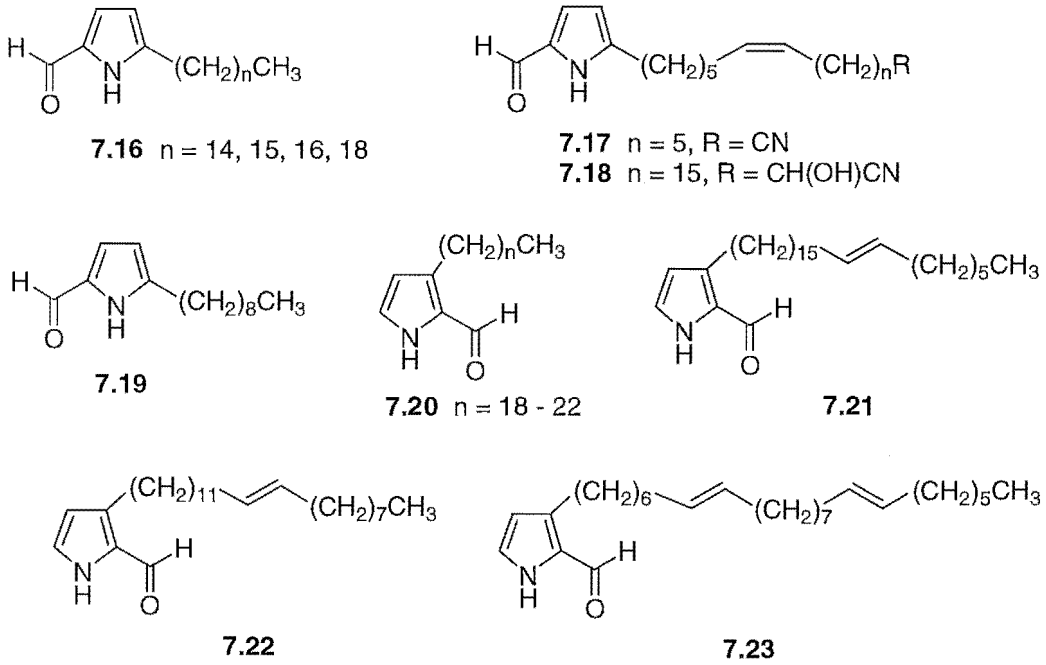



**Figure 7.3.** Alkylpyrrole-2-carboxaldehydes isolated from marine sources.

Despite some similarities with the above compounds, the mycalazals 1 – 2 (Figure 7.2) were the first reported 5-alkylpyrrole-2-carboxaldehydes to possess an extended conjugated unsaturated side chain. No cytotoxicity data has been reported on the 5-alkylpyrrole-2-carboxaldehydes from the earlier studies.

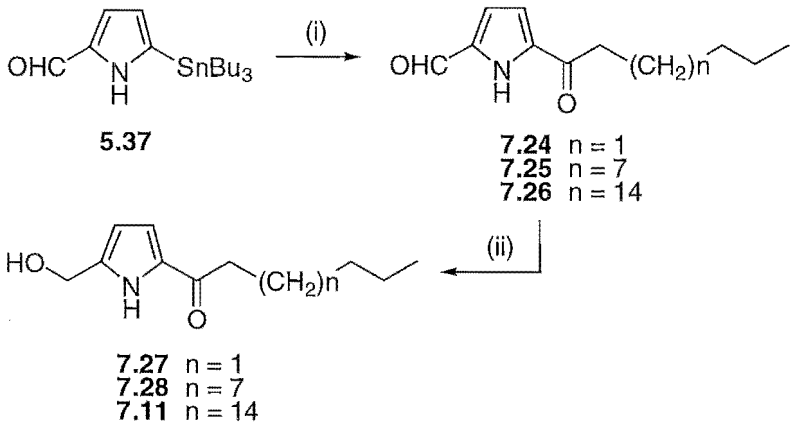
The aim of the research undertaken in this chapter was to synthesise a representative example of the mycalazol natural products, along with some simple 5-acyl-

2-hydroxymethylpyrrole analogues differing in alkyl chain length, by utilising our newly developed Stille coupling methodology (see Chapter 5 for a discussion of this method). The *in vitro* P388 cytotoxicity of these compounds, together with a 5-carboxamido-2-hydroxymethylpyrrole analogue, would then be assayed, and from these results a preliminary understanding of the structure-activity relationship of these compounds could be developed.

## *7.2. Synthesis of Mycalazol 11 and related hydroxymethylpyrroles*

The mycalazol which was deemed to be the most suitable synthetic target in this investigation was the fully saturated acyl side chain example, mycalazol 11 **7.11**. This was due to the ready commercial availability of the parent fatty acid, nonadecanoic acid, and to allow a direct comparison with other synthesised analogues which also contained a fully saturated acyl or carboxamido side chain.

The synthesis of the 5-acyl-2-hydroxymethylpyrroles **7.27**, **7.28** and **7.11** began with the preparation of 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde **5.37** from pyrrole-2-carboxaldehyde, as detailed in Chapter 5. This stannylpyrrole **5.37** was then coupled with either *n*-hexanoyl chloride, lauroyl chloride or nonadecanoyl chloride[7] using bis(triphenylphosphine)palladium(II) dichloride in refluxing toluene to give the 5-acylpyrrole-2-carboxaldehydes **7.24 – 7.26** in yields of 77%, 91% and 92%, respectively (Scheme 7.1). The acylation occurred exclusively at the 5-position under these conditions, with no *N*-acylated pyrrole side-products being detected by $^1$H NMR spectroscopy. The synthesis was then completed by simply reducing the formyl group of **7.24 – 7.26** with zinc borohydride to give the corresponding 5-acyl-2-hydroxymethylpyrroles **7.27**, **7.28** and **7.11** in high yields (93%, 84% and 93%, respectively). The $^1$H NMR data obtained for **7.11** was identical to that reported for the natural product, mycalazol 11.[2]




**5.37**

**7.24** n = 1
**7.25** n = 7
**7.26** n = 14

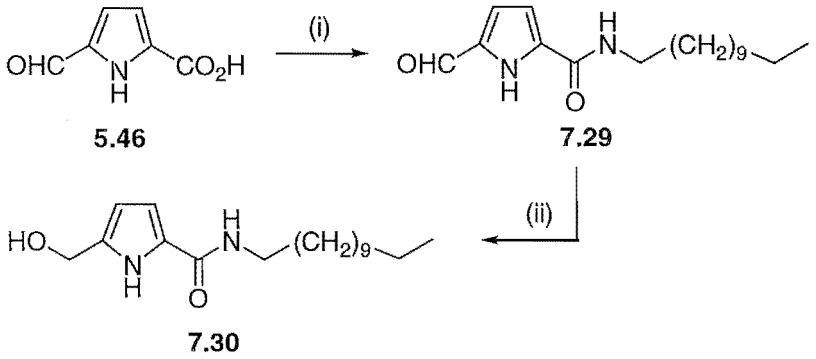
**7.27** n = 1
**7.28** n = 7
**7.11** n = 14

**Scheme 7.1.** *Reagents and conditions*: (i) $CH_3(CH_2)_4COCl$ or $CH_3(CH_2)_{10}COCl$ or $CH_3(CH_2)_{17}COCl$, $(Ph_3P)_2PdCl_2$, PhMe, reflux, 1½ h then KF, MeCN, rt, 1 h (**7.24**, 77%), (**7.25**, 91%), (**7.26**, 92%); (ii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (**7.27**, 93%), (**7.28**, 84%), (**7.11**, 93%).

An important feature of our synthetic methodology is that it allows for the efficient coupling of a stannylpyrrole with an acyl group without the need for protection of the pyrrole nitrogen. Previously reported examples of palladium-catalysed cross-coupling reactions of a stannylpyrrole and an acyl, aryl, heteroaryl or alkyl halide have employed methyl,[8] *tert*-butoxycarbonyl (BOC)[8c,9] or tosyl[10] substitution of the pyrrole nitrogen for protection and/or stability reasons. Another point to note is that a synthesis of the 5-(1-oxohexyl)pyrrole-2-carboxaldehyde **7.24** has previously been reported by Muchowski and Hess using anion chemistry.[11] In this method, a two step, one-pot lithiation sequence of the 6-dimethylamino-1-azafulvene dimer, followed by hydrolysis with sodium acetate in refluxing aqueous tetrahydrofuran, gave the desired pyrrole **7.24** in 54% yield. In contrast, our Stille coupling methodology allows for a greater range of acyl substituents, with a broad range of functionality, to be incorporated onto the parent pyrrole compound due to the avoidance of these bases in the acylation sequence.

The related 5-carboxamido-2-hydroxymethylpyrrole **7.30** was also prepared for testing against the P388 murine leukemia cell line. This compound enabled a comparison of the activity of hydroxymethylpyrrole derivatives bearing an amide side chain (*ie* compound **7.30**) and an acyl side chain (*ie* compounds **7.27**, **7.28** and **7.11**). The

synthesis of **7.30** was achieved by initially coupling 5-formylpyrrole-2-carboxylic acid **5.46**, prepared as detailed in Chapter 5, with dodecylamine, using standard EDCI-coupling methodology,[12] to give the 5-carboxamidopyrrole-2-carboxaldehyde **7.29** in 92% yield (Scheme 7.2). Reduction of the formyl group of **7.29** with zinc borohydride then gave the desired hydroxymethylpyrrole **7.30** in 91% yield.




**5.46** **7.29** **7.30**

**Scheme 7.2.** *Reagents and conditions*: (i) EDCI, HOBt, dodecylamine (92%); (ii) $Zn(BH_4)_2$, $Et_2O$, 0 °C (91%).

### *7.3. P388 Cytotoxicity of mycalazol analogues*

Compounds **7.27**, **7.28**, **7.11** and **7.30** were assayed for *in vitro* cytotoxicity against the P388 murine leukemia cell line. The $ID_{50}$ results of this assay, as summarised in Table 7.2, showed that the most active compounds were the long acyl chain derivatives **7.28** and **7.11**, which had activities of 21 and 24 μg $mL^{-1}$, respectively. These were followed in turn by the carboxamido derivative **7.30** and the short acyl chain derivative **7.27**, which had activities of 52 and 78 μg $mL^{-1}$, respectively. The lower the $ID_{50}$ value for a compound, the more potent its cytotoxicity. These preliminary results suggest that an increased chain length leads to greater activity, although it should be noted that compounds **7.28** and **7.11** have comparable activity (see Table 7.2). Mycalazol 11 **7.11** has previously been reported to have an $ED_{50}$ of 2.5 μg $mL^{-1}$ against the P388 cell line,[2]

while in our present study a 10-fold decrease in activity was recorded. However, the decreased activity of **7.11** in our assay may have been due to the observed formation of a precipitate in the reaction wells of this compound during the P388 assay. Attempts at changing the polarity of the assay solvent in order to prevent this precipitation of **7.11** were unsuccessful, and instead resulted in a loss of activity ($ID_{50}$ > 125 μg $mL^{-1}$). From the assay results detailed in Table 7.2 it would also appear that an acyl side chain leads to increased biological activity relative to the carboxamido side chain, with **7.28** having 2.5-fold greater activity compared to **7.30**, despite similar chain lengths.

| **Compound** | **7.27** | **7.28** | **7.11** | **7.30** |
|---|---|---|---|---|
| Number of atoms in side chain | 6 | 12 | 19 | 14 |
| $ID_{50}$ (μg $mL^{-1}$)† | 78 | 21 | 24# | 52 |

**Table 7.2.** Cytotoxicity data ($ID_{50}$, μg $mL^{-1}$)† of the hydroxymethylpyrroles **7.27**, **7.28**, **7.11**# and **7.30**.

† The lower the value of $ID_{50}$ the more cytotoxic the compound

# A precipitate was observed to form in the reaction wells of **7.11** during the P388 assay.

## *7.4. Summary and future work*

A new and general synthesis of 5-acylpyrrole-2-carboxaldehydes has been developed which utilises a Stille coupling reaction between 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde **5.37** and a (fatty) acid chloride. This coupling proceeds in good yield and without the need to protect either the α-formyl group or the pyrrole nitrogen. This methodology was used to prepare a series of 5-acyl-2-hydroxymethylpyrroles, including the previously reported natural product mycalazol 11 **7.11**. These compounds, together with a 5-carboxamido-2-hydroxymethylpyrrole, were assayed for *in vitro* cytotoxicity against the P388 murine leukemia cell line. The $ID_{50}$ results of this assay indicated that, in general, an increased chain length leads to greater biological activity, and that an acyl side chain has greater activity relative to a carboxamido side chain.

Future work in this area will be centred on the synthesis and P388 assay of further examples of these compounds, incorporating either an acyl chain with varying degrees of unsaturation, or a polypeptide chain, in order to further explore the key features of this class of pyrrole-based compound that are responsible for the biological activity.

## 7.5. References for chapter seven


1 For some general reviews of marine natural products see: (a) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 113; (b) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259; (c) Albizati, K. F.; Martin, V. A.; Agharahimi, M. R.; Stolze, D. A. *Bioorganic Marine Chemistry.* Scheuer, P. J. Ed. Springer-Verlag: Berlin, 1992, Vol 6.

2 Ortega, M. J.; Zubia, E.; Carballo, J. L.; Salvá, J. *Tetrahedron* **1997**, *53*, 331.

3 Tada, H.; Tozyo, T. *Chem. Lett.* **1988**, 803.

4 Stierle, D. B.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 4980.

5 Bowden, B. F.; Clezy, P. S.; Coll, J. C.; Ravi, B. N.; Tapiolas, D. M. *Aust. J. Chem.* **1984**, *37*, 227.

6 Cimino, G.; de Stefano, S.; Minale, L. *Experientia* **1975**, *31*, 1387.

7 Acid chlorides prepared by the method of: Bauer, S. T. *Oil and Soap* **1946**, 1.

8 (a) Bailey, T. R. *Tetrahedron Lett.* **1986**, *27*, 4407. (b) Jousseaume, B.; Kwon, H.; Verlhac, J. –B.; Denat, F.; Dubac, J. *Synlett.* **1993**, 117; (c) Dijkstra, H. P.; ten Have, R.; van Leusen, A. M. *J. Org. Chem.* **1998**, *63*, 5332.

9 (a) Martina, S.; Enkelmann, V.; Wegner, G.; Schlüter, A. –D. *Synthesis* **1991**, 613. (b) Martina, S.; Enkelmann, V.; Schlüter, A. –D.; Wegner, G. *Synth. Met.* **1991**, *41*, 403.

10 Wang, J.; Scott, A. I. *Tetrahedron Lett.* **1996**, *37*, 3247.

11 Muchowski, J. M.; Hess, P. *Tetrahedron Lett.* **1988**, *29*, 777.

12 Tian, Z. –Q.; Brown, B. B.; Mack, D. P.; Hutton, C. A.; Bartlett, P. A. *J. Org. Chem.* **1997**, *62*, 514.

# CHAPTER EIGHT

# EXPERIMENTAL

# 8.1 General Methods and Experimental Procedures

**Nuclear Magnetic Resonance**

Proton detected NMR spectra were obtained on a Varian Unity 300 spectrometer operating at 300 MHz. Carbon detected NMR spectra were obtained on a Varian XL300 spectrometer operating at 75 MHz. Unless otherwise indicated, spectra were obtained at 23 °C. Other NMR experiments described in this thesis *viz.* NOE and the reverse-detected HSMQC, HSQC and HMBC experiments were all obtained on the Unity 300 spectrometer at 300 MHz. At various stages this instrument was fitted with either a Nalorac Z.spec MID300 3 mm Indirect Detection Probe or a Pulsed Field Gradient MLD driver with a 5mm Indirect Detection Probe. Chemical shifts are reported in parts per million (ppm), on the δ scale, and are referenced to the appropriate solvent peaks: $CDCl_3$ referenced to $(CH_3)_4Si$ at $\delta_H$ 0 ppm ($^1H$) and $CDCl_3$ at $\delta_C$ 77.0 ppm ($^{13}C$); $CD_3OD$ referenced to $CHD_2OD$ at $\delta_H$ 3.30 ppm ($^1H$) and $CD_3OD$ at $\delta_C$ 49.3 ppm ($^{13}C$); acetone-$d_6$ referenced to $(CD_3)(CHD_2)CO$ $\delta_H$ 2.17 ($^1H$) and $(CD_3)_2CO$ $\delta_C$ 29.2 ($^{13}C$); $CD_3CN$ referenced to $CHD_2CN$ $\delta_H$ 2.00 ($^1H$) and $CD_3CN$ $\delta_C$ 1.3 ($^{13}C$); DMSO-$d_6$ referenced to $(CD_3)(CHD_2)SO$ $\delta_H$ 2.50 ($^1H$) and $(CD_3)_2SO$ $\delta_C$ 39.6 ($^{13}C$). $^1H$ NMR spectra were obtained using an acquisition time ($A_t$) of 2 s. $^{13}C$ NMR spectra were obtained with an $A_t$ of 0.878 s and typically a delay ($D_1$) of 1 s. All difference NOE experiments were obtained in undegassed solutions, with an acquisition time ($A_t$) of 1 s and an irradiation time ($D_2$) of 2 s. The decoupler was offset 10,000 Hz for the control experiment. HSMQC experiments were obtained with an $A_t$ of 0.2 s, $D_1$ was set for individual samples when setting the null value and $^1J_{CH}$ = 145 Hz. HMQC experiments with the pulsed field gradient system were run with an $A_t$ of 0.137 s, a $D_1$ of 1.0 s and $^1J_{CH}$ = 140 Hz. HMBC experiments were obtained with an $A_t$ of 0.21 s (0.137 s with the pulsed field gradient system), a relaxation delay of 0.3 s, $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ = 8.3 Hz, unless otherwise stated.

**Mass Spectrometry**

Mass Spectrometry was performed on a Kratos MS80 Mass Spectrometer operating at 4 kV. Various ionisation techniques were used including Electron Impact (EI) at 70eV and chemical ionisation (CI; $C_4H_{10}$). The softer ionisation technique of Fast Atom Bombardment (FAB) was used where necessary and was performed with an Ion Tech ZN11FN ion gun using Xe as the reagent gas, operating at 8 kV and 2 mA with either NOBA (*m*-nitrobenzyl alcohol), m-b (magic bullet, 50% dithioerythritol/dithiothreitol), or glycerol as the matrix.

**IR Spectroscopy**

IR spectra were obtained using a Shimadzu 8201PC series FTIR interfaced with a Intel 486 PC running Shimadzu's HyperIR software. Spectra were run in a solution of either $CHCl_3$ or $CS_2$.

**UV Spectroscopy**

UV spectra were obtained using a Hewlett Packard 8452A diode array spectrophotometer. Spectra were run in a solution of either $CHCl_3$ or MeOH.

**Melting Points**

Melting points were taken on an Electrothermal® apparatus and are uncorrected.

**Reagents and Solvents**

Unless otherwise indicated, all reactions were conducted in oven- or flame-dried glassware under an atmosphere of dry nitrogen. Analytical thin layer chromatography (TLC) was conducted on aluminium- or plastic-backed Merck Kieselgel $KG60F_{254}$ silica plates. Visualisation was by shortwave ultraviolet light and/or staining with Ehrlich's reagent[1] or potassium permanganate solution. Flash chromatography was performed on Merck Silica 60 following the guidelines given by Still *et al.*[2] Solvents and reagents used in reactions were purified according to well established procedures.[3] Tetrahydrofuran (THF), ether ($Et_2O$) and benzene (PhH) were distilled from sodium benzophenone ketyl immediately prior to use. Pyrrole, dichloromethane ($CH_2Cl_2$), 1,2-dichloroethane (1,2-DCE), triethylamine (TEA), Hünig's base (*N,N*-diisopropylethylamine) and phosphorus oxychloride ($POCl_3$) were all distilled from calcium hydride. Methanol (MeOH) and

ethanol (EtOH) were distilled from iodine-magnesium turnings and stored under $N_2$ or Ar over 4 Å molecular sieves. Organolithiums and Grignard reagents were obtained from Aldrich Chemical Co. or Acros Organics. Organolithiums and Grignard reagents were titrated before use by the method of Watson and Eastham.[4]

**General Procedure A: Formylation of pyrrole using Vilsmeier-Haack conditions**

Phosphorus oxychloride (1.1 equiv) was added dropwise over 15 min to a solution of *N,N*-dimethylformamide (1.1 equiv) cooled to 10 – 20 °C under $N_2$. 1,2-Dichloroethane (8 mL) was added, followed by a solution of pyrrole (typically 32.3 mmol) in 1,2-dichloroethane (8 mL) over 1 h. The mixture was heated at reflux for 15 min, cooled to 20 °C, treated with a solution of sodium acetate trihydrate (5 equiv) in water (30 mL) and heated at reflux for a further 15 min. The 1,2-dichloroethane layer was separated, and the aqueous phase was extracted with ether (3x15 mL). The combined organic phases were washed with saturated aqueous sodium hydrogencarbonate (3x15 mL), dried and evaporated under reduced pressure. The residue was purified by distillation under high vacuum and/or flash chromatography on silica. See experimental for details.

**General Procedure B: *N*-Acylation of pyrrole using sodium hydride**

To a stirred suspension of sodium hydride (typically 1.26 mmol, 80% suspension in oil washed twice with petroleum ether, 1.2 equiv) in THF (6 mL) under $N_2$ was added the pyrrole (typically 1.09 mmol) dissolved in THF (2 mL). After stirring at rt for 15 min, the electrophile (typically 1.2 – 1.4 equiv) in THF (2 mL) was slowly added and stirring was continued for 1 h at rt. Water (10 mL) was added, the THF was removed under reduced pressure and the aqueous residue was extracted with dichloromethane (3x15 mL). The combined organic phases were washed with saturated aqueous sodium hydrogencarbonate (10 mL), water (10 mL), saturated aqueous brine (10 mL), dried and concentrated under reduced pressure. The residue was purified by flash chromatography on silica. See experimental for details.

**Modification to general procedure B:** The electrophile (typically 1.2 – 1.4 equiv) in THF (2 mL) was slowly added to a cooled mixture (–10 to 0 °C) of the pyrrolyl anion, prepared as described above. Stirring was continued at –10 to 0 °C for 30 min and then

for 1 h at rt, before the reaction mixture was worked up using the same method as described above.

**General Procedure C: *N*-Acylation of pyrrole using 4-dimethylaminopyridine**

To a solution of the pyrrole (typically 0.63 mmol), 4-dimethylaminopyridine (0.1 equiv) and Hünig's base (typically 1.1 – 2.1 equiv) in dichloromethane (8 mL) at rt under $N_2$ was added the electrophile (typically 1.1 – 2.1 equiv), dissolved in dichloromethane (2 mL). The resultant mixture was then stirred for 24 h at rt. Ethyl acetate (10 mL) was added, and the solution was washed with 10% aqueous citric acid (10 mL), water (10 mL), saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. The residue was purified by flash chromatography on silica. See experimental for details.

**General Procedure D: Zinc borohydride reductions**

The *N*-substituted pyrrole-2-carboxaldehyde (typically 0.16 mmol) was dissolved in ether (10 mL) at 0 °C under $N_2$. Zinc borohydride (1 equiv of 0.45 M or 0.14 M solution in ether) was added and the resultant solution was stirred at 0 °C for 30 min. Water (2 mL) and then 10% aqueous glacial acetic acid (2 mL) were carefully added to quench the reaction. The separated aqueous phase was extracted with dichloromethane (2x10 mL) and the combined organic phases were washed with water (2x10 mL), saturated aqueous brine (10 mL), dried and concentrated under reduced pressure. The residue was purified by flash chromatography on silica. See experimental for details.

**General Procedure E: Sodium borohydride reductions**

To a solution of the pyrrole ketone (typically 0.20 mmol) in methanol (10 mL) at 0 °C under $N_2$ was added sodium borohydride (15 equiv). The resulting solution was stirred at 0 °C for 30 min, then diluted with dichloromethane (10 mL), washed with water (2x10 mL), and the organic layer dried and evaporated under reduced pressure. The residue was purified by flash chromatography on silica. See experimental for details.

**General Procedure F: Alpine borane® reductions**

The *N*-substituted pyrrole-2-carboxaldehyde (typically 0.49 mmol) was dissolved in THF (10 mL) at rt under $N_2$. *R*- or *S*-Alpine borane® (1.1 equiv of 0.5 M solution in THF) was added and the resultant solution was stirred at rt for 4 h. The volatiles were

removed under reduced pressure and the resultant oil was purified by flash chromatography on silica. See experimental for details.

**General Procedure G: Camphanate preparation using camphanoyl chloride**

The hydroxymethylpyrrole (typically 0.12 mmol), 4-dimethylaminopyridine (1 equiv) and Hünig's base (1.2 equiv) were dissolved in dichloromethane (8 mL) at rt under $N_2$. (1*S*,4*R*)-(–)-Camphanoyl chloride (1.2 equiv) dissolved in dichloromethane (2 mL) was added and the resultant solution was stirred for 24 h. The solution was extracted with ethyl acetate (10 mL) and the organic phase was washed with 10% aqueous citric acid (10 mL), water (2x10 mL), dried and evaporated under reduced pressure. The resultant oil was purified by flash chromatography on silica. See experimental for details.

**General Procedure H: Camphanate preparation using Mitsunobu conditions**

The hydroxymethylpyrrole (typically 0.11 mmol), triphenylphosphine (1.3 equiv) and (1*S*,4*R*)-(–)-camphanic acid (1.5 equiv) were dissolved in THF (2 mL) and the solution was stirred for 5 min at rt under $N_2$. Diethylazodicarboxylate (1.5 equiv) was added and the resultant solution was stirred for 3 h. The solvent was evaporated under reduced pressure, and the resultant oil was purified by flash chromatography on silica. See experimental for details.

**General Procedure I: Chlorination of hydroxymethylpyrroles**

Methanesulfonyl chloride (1.5 equiv) was added to an ice cooled solution of the hydroxymethylpyrrole (typically 0.38 mmol) in dichloromethane (2 mL) containing Hünig's base (1.5 equiv). Stirring was continued at 0 °C for 20 min and for a further 30 min at rt. The solution was diluted with dichloromethane (10 mL), washed with ice-cold water (10 mL), cold 10% aqueous hydrochloric acid (10 mL), and saturated aqueous sodium hydrogencarbonate (10 mL). The organic phase was then dried and evaporated under reduced pressure. See experimental for details.

**General Procedure J: Hydrolysis of pyrrole esters using sodium hydroxide**

A solution of the pyrrole ester (typically 1.22 mmol) in 50% aqueous methanol (4 mL) containing sodium hydroxide (2.5 equiv) was stirred in a warm water bath (bath temperature 30 – 40 °C), and the progress of the reaction was followed by TLC. After *ca.* 1 h, water (10 mL) was added and the methanol was removed under reduced pressure. The aqueous solution was washed with ether (10 mL), cooled to 5 °C and acidified with 50% aqueous sulfuric acid. The resulting solution was extracted with ether (3x15 mL), and the combined ethereal extracts were washed with saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. See experimental for details.

**General Procedure K: Peptide couplings using 1,3-dicyclohexylcarbodiimide**

A solution of the pyrrole carboxylic acid (typically 0.92 mmol), *L*-leucine methyl ester hydrochloride (1 equiv), Hünig's base (2.1 equiv), and 1-hydroxybenzotriazole hydrate (1 equiv) was stirred in dichloromethane (5 mL) at 0 °C for 10 min under $N_2$. 1,3-Dicyclohexylcarbodiimide (1 equiv) dissolved in dichloromethane (0.5 – 1.5 mL) was added and the mixture was stirred at 0 °C for a further 10 min, and then overnight at rt. The solution was then diluted with dichloromethane (10 mL) and filtered to remove insoluble 1,3-dicyclohexylurea. The organic solution was then washed with 3 M aqueous hydrochloric acid (2x10 mL), water (2x10 mL), dried and evaporated under reduced pressure. The resultant oil was purified by flash chromatography on silica. See experimental for details.

**General Procedure L: Peptide couplings using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride**

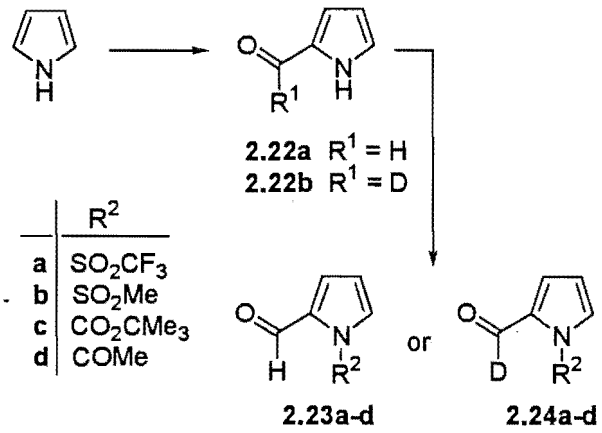
To a stirred solution (~0.1 M) of the pyrrole carboxylic acid (typically 1.14 mmol) and the *L*-amino acid ester hydrochloride (1.1 equiv) in dichloromethane (~11 mL) under $N_2$ at rt were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.3 equiv) and 1-hydroxybenzotriazole hydrate (1.5 equiv). Hünig's base (1.1 equiv) was added and the reaction mixture was stirred for 16 h. The solution was then diluted with dichloromethane (10 mL), washed with 3 M aqueous hydrochloric acid (2x10 mL), water (2x10 mL), dried and evaporated under reduced pressure. The resultant oil was purified by flash chromatography on silica. See experimental for details.

**General Procedure M: Stille coupling of stannylpyrroles**

A stirred mixture of the stannylpyrrole (typically 0.39 mmol), acid chloride (1 equiv) and bis(triphenylphosphine)palladium(II) dichloride (1 mol%) in toluene (20 mL) under $N_2$ was heated at reflux temperature until no starting material was evident by TLC (*ca.* 1½ h). The reaction mixture was allowed to cool to rt, dichloromethane (50 mL) was added, and the resultant solution was washed with water (50 mL), and saturated aqueous brine (50 mL). The organic phase was dried, treated with activated charcoal, filtered and evaporated under reduced pressure. The resulting residue was dissolved in acetonitrile (5 mL), and potassium fluoride (~200 mg) was added. This mixture was then stirred under $N_2$ at rt for 1 h before being filtered through Celite® and evaporated under reduced pressure. The residue was purified by flash chromatography on silica. See experimental for details.

# 8.2 Experimental Work Described in Chapter Two

## 8.2.1. Preparation of unlabelled and [*formyl-d*]-labelled 1-(substituted) pyrrole-2-carboxaldehydes 2.23a-d and 2.24a-d




**[*formyl-d*]-Pyrrole-2-carboxaldehyde 2.22b**

General procedure A was carried out using pyrrole (2.07 mL, 32.3 mmol), $POCl_3$ (3.33 mL, 35.7 mmol, 1.1 equiv) and DMF-$d_7$ (2.00 mL, 35.7 mmol, 1.1 equiv). The resulting residue was distilled under high vacuum and recrystallised from petroleum ether to give **2.22b** (1.64 g, 53%) as a crystalline solid: mp 43 – 44 °C (lit.[5] for unlabelled analogue **2.22a** mp 44 – 45 °C); IR ($CHCl_3$) 3453, 3282, 1642 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 6.36 (m, 1H, pyrrole H4), 7.01 (m, 1H, pyrrole H3), 7.17 (m, 1H, pyrrole H5), 10.09 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 111.3, 122.1, 127.3, 132.7, 179.2 (t, $J$ = 26.6 Hz); HRMS calcd for $C_5H_4DNO$ 96.0434, found 96.0432; Isotopic incorporation 97.6% D.

**1-(Trifluoromethanesulfonyl)pyrrole-2-carboxaldehyde 2.23a and [*formyl-d*]-1-(trifluoromethanesulfonyl)pyrrole-2-carboxaldehyde 2.24a**

To a solution of pyrrole-2-carboxaldehyde **2.22a** (200 mg, 2.10 mmol) in dichloromethane (5 mL) containing Hünig's base (733 μL, 4.21 mmol, 2 equiv), cooled to –78 °C, was added trifluoromethanesulfonic anhydride (637 μL, 3.79 mmol, 1.8 equiv). After 5 min the brown solution was poured onto saturated aqueous sodium

hydrogencarbonate (10 mL). The organic phase, together with subsequent dichloromethane washings (2x10 mL), was dried, and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave **2.23a** (203 mg, 42%) as a yellow oil: IR ($CHCl_3$) 1680, 1175 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 6.63 (m, 1H, pyrrole H4), 7.41 – 7.42 (m, 2H, pyrrole H3 & pyrrole H5), 10.02 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 114.9, 119.0 (q, $J$ = 322.4 Hz), 124.6, 129.5, 135.3, 178.5; HRMS calcd for $C_6H_4F_3NO_3S$ 226.9864, found 226.9864.

The reaction was repeated using [*formyl-d*]-pyrrole-2-carboxaldehyde **2.22b** (102 mg, 1.06 mmol) to give **2.24a** (110 mg, 46%): $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 114.9, 118.9 (q, $J$ = 322.4 Hz), 124.6, 129.5, 135.2, 178.1 (t, $J$ = 28.7 Hz); HRMS calcd for $C_6H_3DF_3NO_3S$ 227.9927, found 227.9924.

**1-(Methanesulfonyl)pyrrole-2-carboxaldehyde 2.23b and [*formyl-d*]-1-(methanesulfonyl)pyrrole-2-carboxaldehyde 2.24b**

General procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (104 mg, 1.09 mmol) and methanesulfonyl chloride (122 μL, 1.58 mmol, 1.4 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **2.23b** (177 mg, 93%) as a pale yellow oil which solidified upon standing: mp 41 – 42 °C (lit.[6] 43 – 44 °C); IR ($CHCl_3$) 1679, 1370, 1174 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.63 (s, 3H, $SO_2$*Me*), 6.42 (m, 1H, pyrrole H4), 7.24 (m, 1H, pyrrole H3), 7.61 (m, 1H, pyrrole H5), 9.68 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 42.6, 111.4, 129.1, 130.4, 132.8, 178.1; HRMS calcd for $C_6H_7NO_3S$ 173.0147, found 173.0146.

The reaction was repeated using [*formyl-d*]-pyrrole-2-carboxaldehyde **2.22b** (102 mg, 1.06 mmol) to give **2.24b** (170 mg, 92%): $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 42.3, 111.2, 128.8, 130.1, 132.4, 177.6 (t, $J$ = 28.2 Hz); HRMS calcd for $C_6H_6DNO_3S$ 174.0209, found 174.0207.

**1-(*tert*-Butoxycarbonyl)pyrrole-2-carboxaldehyde 2.23c and [*formyl-d*]-1-(*tert*-butoxycarbonyl)pyrrole-2-carboxaldehyde 2.24c**

General procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (50 mg, 0.53 mmol) and BOC-ON® (129 mg, 0.53 mmol, 1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:8) gave **2.23c** (94 mg, 91%) as a colourless oil:[7]

IR ($CHCl_3$) 1747, 1661 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.65 (s, 9H, C*Me*$_3$), 6.29 (m, 1H, pyrrole H4), 7.19 (m, 1H, pyrrole H3), 7.45 (m, 1H, pyrrole H5), 10.33 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 27.9, 85.7, 111.6, 121.1, 127.3, 134.7, 148.3, 182.3; HRMS calcd for $C_{10}H_{13}NO_3$ 195.0895, found 195.0898.

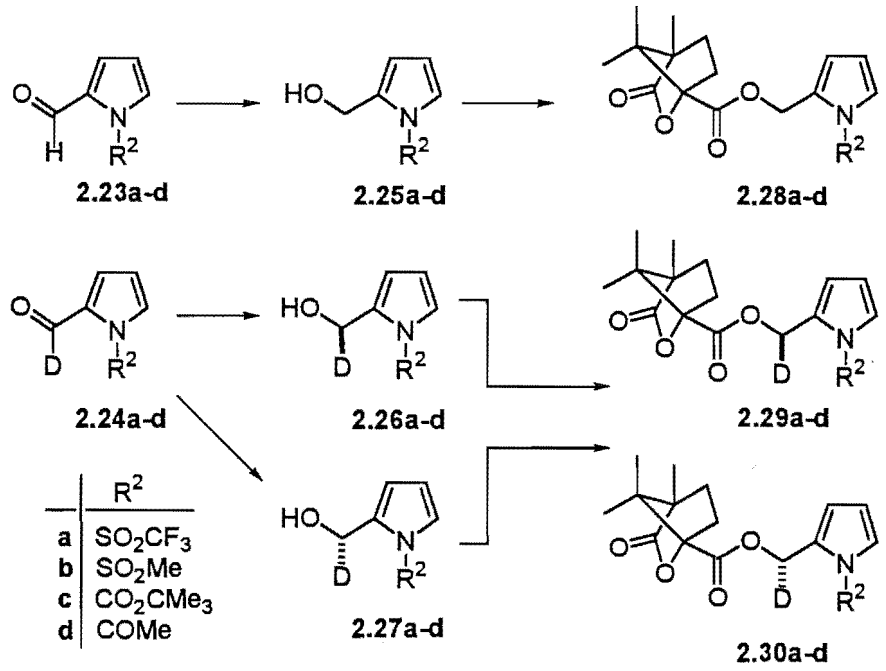
The reaction was repeated using [*formyl-d*]-pyrrole-2-carboxaldehyde **2.22b** (60 mg, 0.62 mmol) to give **2.24c** (106 mg, 87%): $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 27.8, 85.6, 111.6, 121.0, 127.2, 132.8, 148.2, 181.8 (t, $J$ = 28.2 Hz); HRMS calcd for $C_{10}H_{12}DNO_3$ 196.0958, found 196.0958.

**1-(Acetyl)pyrrole-2-carboxaldehyde 2.23d and [*formyl-d*]-1-(acetyl)pyrrole-2-carboxaldehyde 2.24d**

General procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (105 mg, 1.10 mmol) and acetyl chloride (112 μL, 1.60 mmol, 1.5 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **2.23d** (112 mg, 74%) as a yellow solid: mp 74 – 75 °C (lit.[8] 76 – 78 °C); IR ($CHCl_3$) 1732, 1663 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.67 (s, 3H, CO*Me*), 6.37 (m, 1H, pyrrole H4), 7.23 (m, 1H, pyrrole H3), 7.34 (m, 1H, pyrrole H5), 10.31 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 23.6, 112.6, 122.3, 126.6, 135.3, 169.0, 182.3; HRMS calcd for $C_7H_7NO_2$ 137.0477, found 137.0475.

The reaction was repeated using [*formyl-d*]-pyrrole-2-carboxaldehyde **2.22b** (30 mg, 0.31 mmol) to give returned **2.22b** (6 mg) and **2.24d** (33 mg, 94% based on returned starting material): $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 23.7, 112.7, 122.4, 126.6, 135.3, 169.1, 182.1 (t, $J$ = 28.2 Hz); HRMS calcd for $C_7H_6DNO_2$ 138.0540, found 138.0536.

## 8.2.2. Preparation of unlabelled and [*methylene-d*$_1$]-labelled camphanates 2.28a-d, 2.29a-d and 2.30a-d




**2-Hydroxymethyl-1-(trifluoromethanesulfonyl)pyrrole 2.25a**

The *N*-triflylpyrrole **2.23a** (197 mg, 0.87 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **2.25a** (178 mg, 90%) as a colourless oil: IR ($CHCl_3$) 3605, 1418, 1177 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.55 (bs, 1H, O*H*), 4.70 (s, 2H, C$H_2$OH), 6.40 (m, 1H, pyrrole H4), 6.47 (m, 1H, pyrrole H3), 7.11 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 56.5, 114.2, 117.0, 119.2 (q, $J$ = 322.4 Hz), 124.5, 136.5; HRMS calcd for $C_6H_6F_3NO_3S$ 229.0021, found 229.0018.

**2-Hydroxymethyl-1-(methanesulfonyl)pyrrole 2.25b.**

The *N*-mesylpyrrole **2.23b** (31 mg, 0.18 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **2.25b** (29 mg, 91%) as a pale orange solid. An analytical sample was obtained by sublimation under reduced pressure to give a white crystalline solid: mp 67 – 68 °C; IR ($CHCl_3$) 3587, 1367, 1178 $cm^{-1}$; $^1H$ NMR ($CDCl_3$,

300 MHz) δ 3.30 (s, 3H, $SO_2Me$), 4.77 (s, 2H, $CH_2OH$), 6.25 (m, 1H, pyrrole H4), 6.31 (m, 1H, pyrrole H3), 7.16 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 42.8, 56.3, 111.1, 115.2, 123.1, 133.5; HRMS calcd for $C_6H_9NO_3S$ 175.0303, found 175.0303. Anal. Calcd for $C_6H_9NO_3S$: C, 41.13; H, 5.18; N, 7.99. Found: C, 41.11; H, 5.24; N, 8.30.

**2-Hydroxymethyl-1-(*tert*-butoxycarbonyl)pyrrole 2.25c**

The *N*-BOC-pyrrole **2.25c** (26 mg, 0.13 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:5) gave **2.25c** (24 mg, 91%) as a colourless oil: IR ($CHCl_3$) 3541, 1728, 1342 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.61 (s, 9H, $CMe_3$), 3.63 (bs, 1H, O*H*), 4.64 (s, 2H, $CH_2OH$), 6.09 (m, 1H, pyrrole H4), 6.17 (m, 1H, pyrrole H3), 7.16 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 27.9, 57.6, 84.5, 110.4, 113.5, 121.9, 134.8, 150.0; HRMS calcd for $C_{10}H_{15}NO_3$ 197.1052, found 197.1050.

**2-Hydroxymethyl-1-(acetyl)pyrrole 2.25d**

The *N*-acetylpyrrole **2.23d** (31 mg, 0.22 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **2.25d** (31 mg, 98%) as a yellow solid: mp 83 – 84 °C; IR ($CHCl_3$) 3535, 1705, 1323 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.57 (s, 3H, CO*Me*), 4.62 (s, 2H, $CH_2OH$), 6.19 – 6.22 (m, 2H, pyrrole H4 & pyrrole H3), 7.08 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 23.5, 57.6, 112.0, 114.5, 121.6, 135.2, 170.4; HRMS calcd for $C_7H_9NO_2$ 139.0633, found 139.0634.

**(1*S*,4*R*)-1-(Trifluoromethanesulfonyl)pyrrol-2-ylmethyl camphanate 2.28a**

General procedure G was carried out using the *N*-triflylhydroxymethylpyrrole **2.25a** (27 mg, 0.12 mmol). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave **2.28a** (46 mg, 95%) as a colourless oil: IR ($CHCl_3$) 1786, 1751, 1421, 1177 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 3H, camph-Me), 1.05 (s, 3H, camph-Me), 1.11 (s, 3H, camph-Me), 1.68 (m, 1H, camph-$CH_2$), 1.88 – 2.08 (m, 2H, camph-$CH_2$), 2.43 (m, 1H, camph-$CH_2$), 5.32 (ABq, $J$ = 14.4 Hz, 1H, $CH_2O$-camph), 5.37 (ABq, $J$ = 14.4 Hz, 1H, $CH_2O$-camph), 6.46 (m, 1H, pyrrole H4), 6.65 (m, 1H, pyrrole H3), 7.20 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 9.5, 16.4, 16.5, 28.8, 30.6, 54.2, 54.7,

57.8, 90.8, 114.2, 119.0 (q, $J$ = 322.4 Hz), 120.3, 125.6, 130.0, 166.9, 177.9; HRMS calcd for $C_{16}H_{18}F_3NO_6S$ 409.0807, found 409.0812.

**(1*S*,4*R*)-1-(Methanesulfonyl)pyrrol-2-ylmethyl camphanate 2.28b**

General procedure G was carried out using the *N*-mesylhydroxymethylpyrrole **2.25b** (15 mg, 0.09 mmol). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **2.28b** (27 mg, 86%) as a colourless oil which solidified upon standing. An analytical sample was obtained by recrystallisation from petroleum ether: mp 73 – 74 °C; IR ($CHCl_3$) 1788, 1736, 1369, 1182, 1169 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 3H, camph-Me), 1.09 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.68 (m, 1H, camph-$CH_2$), 1.90 – 2.06 (m, 2H, camph-$CH_2$), 2.46 (m, 1H, camph-$CH_2$), 3.37 (s, 3H, $SO_2Me$), 5.35 (ABq, $J$ = 12.7 Hz, 1H, $CH_2O$-camph), 5.42 (ABq, $J$ = 12.7 Hz, 1H, $CH_2O$-camph), 6.28 (m, 1H, pyrrole H4), 6.44 (m, 1H, pyrrole H3), 7.23 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 9.4, 16.4, 16.5, 28.6, 30.7, 42.9, 54.3, 54.6, 58.9, 90.8, 111.1, 117.7, 124.3, 127.2, 166.8, 177.8; HRMS calcd for $C_{16}H_{21}NO_6S$ 355.1090, found 355.1094; Anal. Calcd for $C_{16}H_{21}NO_6S$: C, 54.07; H, 5.96; N, 3.94. Found: C, 54.06; H, 5.84; N, 4.05.

**(1*S*,4*R*)-1-(*tert*-Butoxycarbonyl)pyrrol-2-ylmethyl camphanate 2.28c**

General procedure G was carried out using the *N*-BOC-hydroxymethylpyrrole **2.25c** (21 mg, 0.11 mmol). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **2.28c** (30 mg, 76%) as a colourless oil which solidified upon standing to give a white solid. An analytical sample was obtained by recrystallisation from petroleum ether: mp 104 – 105 °C; IR ($CHCl_3$) 1784, 1749, 1346, 1319 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.93 (s, 3H, camph-Me), 1.02 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.59 (s, 9H, $CMe_3$), 1.66 (m, 1H, camph-$CH_2$), 1.90 (m, 1H, camph-$CH_2$), 2.03 (m, 1H, camph-$CH_2$), 2.43 (m, 1H, camph-$CH_2$), 5.41 (ABq, $J$ = 13.0 Hz, 1H, $CH_2O$-camph), 5.45 (ABq, $J$ = 13.0 Hz, 1H, $CH_2O$-camph), 6.14 (m, 1H, pyrrole H4), 6.34 (m, 1H, pyrrole H3), 7.28 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 9.6, 16.5, 16.6, 27.9, 28.9, 30.6, 54.1, 54.7, 60.2, 84.1, 91.1, 110.1, 116.3, 123.0, 127.9, 148.6, 167.1, 178.1; HRMS calcd for $C_{20}H_{27}NO_6$ 377.1838, found 377.1845; Anal. Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.37; H, 7.01; N, 3.43.

**(1*S*,4*R*)-1-(Acetyl)pyrrol-2-ylmethyl camphanate 2.28d**

General procedure G was carried out using the *N*-acetylhydroxymethylpyrrole **2.25d** (27 mg, 0.19 mmol). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **2.28d** (61 mg, 99%) as a colourless oil which solidified upon standing. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether: mp 85 – 86 °C; IR ($CHCl_3$) 1784, 1726, 1310 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 3H, camph-Me), 1.04 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.67 (m, 1H, camph-$CH_2$), 1.92 (m, 1H, camph-$CH_2$), 2.04 (m, 1H, camph-$CH_2$), 2.44 (m, 1H, camph-$CH_2$), 2.56 (s, 3H, CO*Me*), 5.44 (ABq, $J$ = 13.5 Hz, 1H, C*H*$_2$O-camph), 5.49 (ABq, $J$ = 13.5 Hz, 1H, C*H*$_2$O-camph), 6.24 (m, 1H, pyrrole H4), 6.37 (m, 1H, pyrrole H3), 7.15 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 9.4, 16.3, 16.4, 23.3, 28.7, 30.5, 53.9, 54.5, 60.6, 90.9, 111.4, 116.2, 122.3, 128.6, 166.8, 168.5, 178.0; HRMS calcd for $C_{17}H_{21}NO_5$ 319.1420, found 319.1421; Anal. Calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39. Found: C, 64.23; H, 6.79; N, 4.27.

**[*methylene-d*$_1$]-(1*S*,4*R*)-1-(Trifluoromethanesulfonyl)pyrrol-2-ylmethyl camphanate 2.29a and 2.30a**

***S*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-triflylpyrrole **2.24a** (30 mg, 0.13 mmol) and *S*-Alpine borane® (0.29 mL of 0.5 M solution in THF, 0.14 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a mixture of **2.26a** and **2.27a** (~9:1 from analysis of camphanates prepared by method G below) which was subsequently used without further purification: $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.64 (bs, 1H, O*H*), 4.68 (s, 1H, C*H*DOH), 6.40 (m, 1H, pyrrole H4), 6.47 (m, 1H, pyrrole H3), 7.12 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 56.2 (t, $J$ = 23.0 Hz), 114.2, 117.0, 119.2 (q, $J$ = 322.4 Hz), 124.5, 136.5; HRMS calcd for $C_6H_5DF_3NO_3S$ 230.0083, found 230.0086.

The preceding sample of **2.26a** and **2.27a** (12 mg, 0.05 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (14 mg, 0.06 mmol, 1.2 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **2.29a** and **2.30a** (9 mg, 40%, ~9:1) as a colourless oil: $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 3H, camph-Me), 1.05 (s, 3H, camph-Me), 1.11 (s, 3H, camph-Me), 1.68 (m, 1H, camph-$CH_2$), 1.89 – 2.08 (m, 2H, camph-$CH_2$), 2.43 (m, 1H, camph-$CH_2$), 5.31

(s, 0.9H, C*H*DO- **2.29a**), 5.33 (s, 0.1H, C*H*DO- **2.30a**), 6.46 (m, 1H, pyrrole H4), 6.65 (m, 1H, pyrrole H3), 7.20 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.5, 16.4, 16.5, 28.8, 30.6, 54.2, 54.7, 57.5 (t, $J$ = 22.9 Hz), 90.8, 114.2, 119.0 (q, $J$ = 322.4 Hz), 120.3, 125.6, 130.0, 166.9, 177.9; HRMS calcd for $C_{16}H_{17}DF_3NO_6S$ 410.0870, found 410.0863.

A further sample of **2.26a** and **2.27a** (13 mg, 0.06 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (17 mg, 0.08 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave unreacted **2.26a** and **2.27a** (5 mg) and a mixture of **2.29a** and **2.30a** (4 mg, 31% based on returned starting material, ~1:5) as a colourless oil. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.31 (s, 0.17H, C*H*DO- **2.29a**), 5.33 (s, 0.83H, C*H*DO- **2.30a**).

***R*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-triflylpyrrole **2.24a** (37 mg, 0.16 mmol) and *R*-Alpine borane® (0.37 mL of 0.5 M solution in THF, 0.18 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **2.26a** and **2.27a** (1:19 from an analysis of camphanates prepared by method G below) which was subsequently used without further purification. Spectral data as recorded above.

The preceding sample of **2.26a** and **2.27a** (14 mg, 0.06 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (16 mg, 0.08 mmol, 1.2 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **2.29a** and **2.30a** (13 mg, 53%, 1:19) as a colourless oil. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.31 (s, 0.05H, C*H*DO- **2.29a**), 5.33 (s, 0.95H, C*H*DO- **2.30a**).

A further sample of **2.26a** and **2.27a** (10 mg, 0.04 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (13 mg, 0.07 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave unreacted **2.26a** and **2.27a** (2 mg) and a mixture of **2.29a** and **2.30a** (5 mg, 32% based on returned starting material ~6:1) as a colourless oil. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.31 (s, 0.86H, C*H*DO- **2.29a**), 5.33 (s, 0.14H, C*H*DO- **2.30a**).

**[*methylene-d*$_1$]-(1*S*,4*R*)-1-(Methanesulfonyl)pyrrol-2-ylmethyl camphanate 2.29b and 2.30b**

***S*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-mesylpyrrole **2.24b** (50 mg, 0.28 mmol) and *S*-Alpine borane® (0.63 mL of 0.5 M solution in THF, 0.31 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a mixture of **2.26b** and **2.27b** (37 mg, 73%, ~9:1 from analysis of camphanates prepared by method G below) as a pale yellow solid: $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.30 (s, 3H, $SO_2$*Me*), 4.72 (s, 1H, C*H*DOH), 6.22 (m, 1H, pyrrole H4), 6.29 (m, 1H, pyrrole H3), 7.14 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 42.7, 55.9 (t, $J$ = 21.9 Hz), 111.0, 115.1, 123.0, 133.4; HRMS calcd for $C_6H_8DNO_3S$ 176.0366, found 176.0365.

The preceding sample of **2.26b** and **2.27b** (10 mg, 0.06 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (16 mg, 0.08 mmol, 1.4 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **2.29b** and **2.30b** (10 mg, 53%, ~9:1) as a white solid: $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 3H, camph-Me), 1.09 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.68 (m, 1H, camph-$CH_2$), 1.90 – 2.06 (m, 2H, camph-$CH_2$), 2.46 (m, 1H, camph-$CH_2$), 3.37 (s, 3H, $SO_2$*Me*), 5.35 (s, 0.9H, C*H*DO- **2.29b**), 5.41, (s, 0.1H, C*H*DO- **2.30b**), 6.28 (m, 1H, pyrrole H4), 6.44 (m, 1H, pyrrole H3), 7.23 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.4, 16.4, 16.5, 28.6, 30.7, 42.9, 54.3, 54.6, 58.7 (t, $J$ = 23.0 Hz), 90.8, 111.1, 117.7, 124.3, 127.2, 166.8, 177.8; HRMS calcd for $C_{16}H_{20}DNO_6S$ 356.1152, found 356.1146.

A further sample of **2.26b** and **2.27b** (10 mg, 0.06 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (18 mg, 0.09 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave a mixture of **2.29b** and **2.30b** (11 mg, 54%, ~2:3) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.35 (s, 0.4H, C*H*DO- **2.29b**), 5.41 (s, 0.6H, C*H*DO- **2.30b**).

***R*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-mesylpyrrole **2.24b** (86 mg, 0.49 mmol) and *R*-Alpine borane® (1.09 mL of 0.5 M solution in THF, 0.54 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a mixture of **2.26b** and **2.27b** (quantitative, ~1:15 from

analysis of camphanates prepared by method G below) as a white solid. Spectral data as recorded above.

The preceding sample of **2.26b** and **2.27b** (35 mg, 0.20 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (53 mg, 0.24 mmol, 1.2 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) gave a mixture of **2.29b** and **2.30b** (22 mg, 31%, ~1:15) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.35 (s, 0.06H, C*H*DO- **2.29b**), 5.41(s, 0.94H, C*H*DO- **2.30b**).

A further sample of **2.26b** and **2.27b** (35 mg, 0.20 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (59 mg, 0.30 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) gave a mixture of **2.29b** and **2.30b** (51 mg, 72%, ~3:2) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.35 (s, 0.6H, C*H*DO- **2.29b**), 5.41 (s, 0.4H, C*H*DO- **2.30b**).

**[*methylene-d*$_1$]-(1*S*,4*R*)-1-(*tert*-Butoxycarbonyl)pyrrol-2-ylmethyl camphanate 2.29c and 2.30c**

***S*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-BOC-pyrrole **2.24c** (34 mg, 0.17 mmol) and *S*-Alpine borane® (0.38 mL of 0.5 M solution in THF, 0.19 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave a mixture of **2.26c** and **2.27c** (~9:1 from analysis of camphanates prepared by method G below) which was subsequently used without further purification: $^1$H NMR ($CDCl_3$, 300 MHz) δ 1.61 (s, 9H, C*Me*$_3$), 3.61 (bs, 1H, O*H*), 4.63 (s, 1H, C*H*DOH), 6.09 (m, 1H, pyrrole H4), 6.17 (m, 1H, pyrrole H3), 7.16 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 27.9, 57.3 (t, $J$ = 21.9 Hz), 84.4, 110.3, 113.4, 121.8, 134.7, 150.0; HRMS calcd for $C_{10}H_{14}DNO_3$ 198.1115, found 198.1114.

The preceding sample of **2.26c** and **2.27c** (17 mg, 0.09 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (22 mg, 0.10 mmol, 1.2 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) followed by recrystallisation from petroleum ether gave a mixture of **2.29c** and **2.30c** (22 mg, 69%, ~9:1) as a white solid: $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.93 (s, 3H, camph-Me), 1.02 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.59 (s, 9H, C*Me*$_3$), 1.66 (m, 1H, camph-$CH_2$), 1.90 (m, 1H, camph-$CH_2$), 2.03 (m, 1H, camph-$CH_2$), 2.43 (m, 1H, camph-$CH_2$), 5.41 (s, 0.9H, C*H*DO- **2.29c**), 5.42 (s, 0.1H, C*H*DO- **2.30c**), 6.14 (m, 1H, pyrrole

H4), 6.34 (m, 1H, pyrrole H3), 7.28 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.6, 16.5, 16.6, 27.9, 28.9, 30.6, 54.1, 54.7, 60.0 (t, $J$ = 23.0 Hz), 84.2, 91.1, 110.1, 116.4, 123.0, 127.9, 148.6, 167.1, 178.2; HRMS calcd for $C_{20}H_{26}DNO_6$ 378.1901, found 378.1911.

A further sample of **2.26c** and **2.27c** (17 mg, 0.09 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (26 mg, 0.13 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:7) followed by recrystallisation from petroleum ether gave a mixture of **2.29c** and **2.30c** (15 mg, 46%, ~4:5) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 0.44H, C*H*DO- **2.29c**), 5.42 (s, 0.56H, C*H*DO- **2.30c**).

***R*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-BOC-pyrrole **2.24c** (30 mg, 0.15 mmol) and *R*-Alpine borane® (0.33 mL of 0.5 M solution in THF, 0.17 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:5) gave a mixture of **2.26c** and **2.27c** (1:19 from analysis of camphanates prepared by method C below) which was subsequently used without further purification. Spectral data as recorded above.

The preceding sample of **2.26c** and **2.27c** (15 mg, 0.08 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (20 mg, 0.09 mmol, 1.2 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave a mixture of **2.29c** and **2.30c** (24 mg, 82%, 1:19) as a white solid: Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 0.05H, C*H*DO- **2.29c**), 5.42 (s, 0.95H, C*H*DO- **2.30c**).

A further sample of **2.26c** and **2.27c** (15 mg, 0.08 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (23 mg, 0.11 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) followed by recrystallisation from petroleum ether gave a mixture of **2.29c** and **2.30c** (10 mg, 36%, ~5:4) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 0.56H, C*H*DO- **2.29c**), 5.42 (s, 0.44H, C*H*DO- **2.30c**).

**[*methylene-d*$_1$]-(1*S*,4*R*)-1-(Acetyl)pyrrol-2-ylmethyl camphanate 2.29d and 2.30d**

***S*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-acetylpyrrole **2.24d** (31 mg, 0.23 mmol) and *S*-Alpine borane® (0.50 mL of 0.5 M solution in THF, 0.25 mmol, 1.1 equiv). Flash chromatography on silica (ethyl

acetate/petroleum ether, 1:1) gave a mixture of **2.26d** and **2.27d** (~9:1 from analysis of camphanates prepared by method G below) which was subsequently used without further purification: $^1$H NMR ($CDCl_3$, 300 MHz) δ 2.58 (s, 3H, CO*Me*), 4.61 (s, 1H, C*H*DOH), 6.19 – 6.23 (m, 2H, pyrrole H4 & pyrrole H3), 7.08 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 23.6, 57.4 (t, $J$ = 21.9 Hz), 112.1, 114.6, 121.6, 135.2, 170.4; HRMS calcd for $C_7H_8DNO_2$ 140.0696, found 140.0696.

The preceding sample of **2.26d** and **2.27d** (20 mg, 0.14 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (37 mg, 0.17 mmol, 1.2 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a mixture of **2.29d** and **2.30d** (16 mg, 35%, ~9:1) as a white solid: $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 3H, camph-Me), 1.04 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.67 (m, 1H, camph-$CH_2$), 1.92 (m, 1H, camph-$CH_2$), 2.04 (m, 1H, camph-$CH_2$), 2.44 (m, 1H, camph-$CH_2$), 2.56 (s, 3H, CO*Me*), 5.43 (s, 0.9H, C*H*DO- **2.29d**), 5.44 (s, 0.1H, C*H*DO- **2.30d**), 6.24 (m, 1H, pyrrole H4), 6.37 (m, 1H, pyrrole H3), 7.15 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.5, 16.4, 16.5, 23.5, 28.8, 30.6, 54.0, 54.6, 60.5 (t, $J$ = 23.0 Hz), 91.1, 111.6, 116.3, 122.3, 128.8, 167.0, 168.5, 178.1; HRMS calcd for $C_{17}H_{20}DNO_5$ 320.1481, found 320.1478.

A further sample of **2.26d** and **2.27d** (15 mg, 0.11 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (32 mg, 0.16 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a mixture of **2.29d** and **2.30d** (15 mg, 43%, ~1:1) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.43 (s, 0.5H, C*H*DO- **2.29d**), 5.44 (s, 0.5H, C*H*DO- **2.30d**).

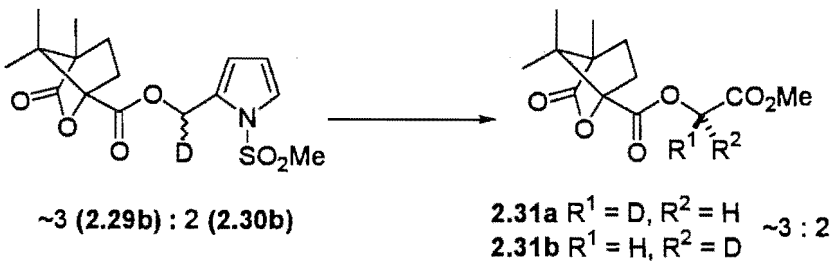
***R*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-acetylpyrrole **19d** (27 mg, 0.20 mmol) and *R*-Alpine borane® (0.43 mL of 0.5 M solution in THF, 0.21 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:5) gave a mixture of **2.26d** and **2.27d** (23 mg, 85%, 1:19 from analysis of camphanates prepared by method G below) as a pale yellow solid. Spectral data as recorded above.

The preceding sample of **2.26d** and **2.27d** (13 mg, 0.09 mmol) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (26 mg, 0.12 mmol, 1.4 equiv) according to general procedure G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a

mixture of **2.29d** and **2.30d** (3 mg, 11%, 1:19) as a white solid: Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.43 (s, 0.05H, C*H*DO- **2.29d**), 5.44 (s, 0.95H, C*H*DO- **2.30d**).

A further sample of **2.26d** and **2.27d** (10 mg, 0.07 mmol) was reacted with (1*S*,4*R*)-(–)-camphanic acid (23 mg, 0.11 mmol, 1.5 equiv) according to general method H. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave a mixture of **2.29d** and **2.30d** (18 mg, 77%, ~1:1) as a white solid. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.43 (s, 0.5H, C*H*DO- **2.29d**), 5.44 (s, 0.5H, C*H*DO- **2.30d**).

## 8.2.3 Ozonolysis of [*methylene-d*$_1$]-(1*S*,4*R*)-1-(methanesulfonyl)pyrrol-2-ylmethyl camphanate 2.29b and 2.30b




~3 (**2.29b**) : 2 (**2.30b**)

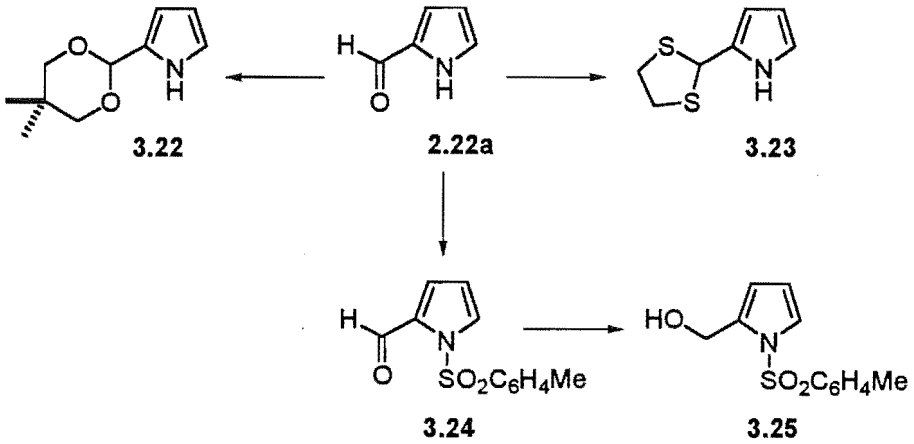
**2.31a** $R^1$ = D, $R^2$ = H
**2.31b** $R^1$ = H, $R^2$ = D
~3 : 2

**Methyl [*methylene-d*$_1$]-(1*S*,4*R*)-camphanoylglycolate 2.31a and 2.31b**

A mixture of **2.29b** and **2.30b** (17 mg, 0.05 mmol, ~3:2) was dissolved in dichloromethane and loaded onto silica (1 g, 50 – 100 mesh) by evaporation under reduced pressure. The residue was cooled to –78 °C and ozone was bubbled through the silica until a blue colour persisted (*ca.* 20 min). After warming to rt, the products were eluted from the silica with ethyl acetate. The solvent was removed under reduced pressure and the residue was dissolved in methanol (10 mL) and treated with an excess of diazomethane. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave a mixture of **2.31a** and **2.31b** (5 mg, 39%, ~3:2) as a colourless oil:[9] IR ($CHCl_3$) 1788, 1727, 1353 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 1.04 (s, 3H, camph-Me), 1.11 (s, 3H, camph-Me), 1.14 (s, 3H, camph-Me), 1.73 (m, 1H, camph-$CH_2$), 1.98 (m, 1H, camph-$CH_2$), 2.10 (m, 1H, camph-$CH_2$), 2.48 (m, 1H, camph-$CH_2$), 3.34 (s, 3H, $CO_2$*Me*), 4.76 (s, 0.6H, C*H*DO- **2.31a**), 4.83 (s, 0.4H, C*H*DO- **2.31b**); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.6, 16.6, 16.6, 28.8, 30.8, 41.8, 54.9, 54.9, 62.5 (t, $J$ = 23.0 Hz), 90.8, 165.5, 166.7, 178.0.

# 8.3 Experimental Work Described in Chapter Three

## 8.3.1. Preparation of starting materials




**2-(5,5-Dimethyl-1,3-dioxan-2-yl)-1*H*-pyrrole 3.22**

The formyl protected pyrrole **3.22** was prepared by the method described by Loader and Anderson.[10] Spectral data were consistent with that reported by Loader and Anderson: mp 61 °C (lit.[10] mp 62 – 62.5 °C); IR ($CHCl_3$) 3476, 2961, 2855, 1101 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.75 (s, 3H, C*Me*$_2$), 1.23 (s, 3H, C*Me*$_2$), 3.63 (m, 4H, C*H*$_2$), 5.44 (s, 1H, C*H*O), 6.12 (m, 1H, pyrrole H4), 6.21 (m, 1H, pyrrole H3), 6.61 (m, 1H, pyrrole H5), 8.69 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.6, 22.8, 29.9, 77.0, 96.7, 106.0, 108.0, 117.5, 128.5; HRMS calcd for $C_{10}H_{15}NO_2$ 181.1103, found 181.1102.

**2-(1*H*-Pyrrol-2-yl)-1,3-dithiolane 3.23**

To a solution of pyrrole-2-carboxaldehyde **2.22a** (1.00 g, 10.52 mmol) in methanol (10 mL) under $N_2$ was added 1,2-ethanedithiol (0.97 mL, 11.57 mmol, 1.1 equiv). Once all the solid had dissolved, aniline hydrochloride (27 mg, 0.21 mmol, 0.02 equiv) was added, and the solution was stirred at rt for 1 h. The solvent was removed under reduced pressure and the resultant residue was dissolved in toluene (10 mL), washed with water (2x10 mL), 0.5 N aqueous potassium hydroxide (3x10 mL), water (10 mL), dried over sodium sulfate, and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave **3.23** (1.65 g, 92%) as

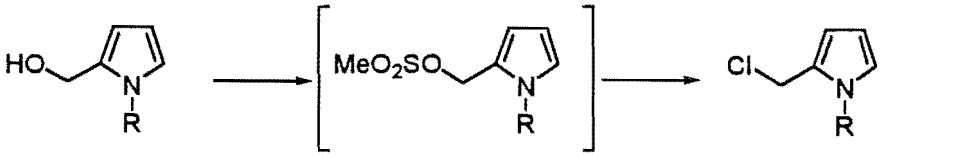
a pale orange solid. An analytical sample was obtained by recrystallisation from petroleum ether to give white crystals: mp 28 °C (lit.[11] mp 27 – 30 °C); IR ($CHCl_3$) 3460, 3030, 3011, 2930 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.35 (m, 4H, SC*H*$_2$), 5.80 (s, 1H, C*H*S), 6.10 (m, 1H, pyrrole H4), 6.18 (m, 1H, pyrrole H3), 6.74 (m, 1H, pyrrole H5), 8.48 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 39.6, 49.3, 107.9, 108.5, 118.4, 128.6; HRMS calcd for $C_7H_9NS_2$ 171.0176, found 171.0176.

**1-[(4-Methylphenyl)sulfonyl]pyrrole-2-carboxaldehyde 3.24**

General procedure B was carried out using pyrrole-2-carboxaldehyde (105 mg, 1.11 mmol) and (4-methylphenyl)sulfonyl chloride (306 mg, 1.60 mmol, 1.4 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **3.24** (272 mg, 99%) as a pink solid: mp 92 – 94 °C (lit.[12] 94 °C); IR ($CHCl_3$) 1670, 1379, 1192, 1175, 1159 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.41 (s, 3H, Ph*Me*), 6.40 (m, 1H, pyrrole H4), 7.16 (m, 1H, pyrrole H3), 7.33 (m, 2H, phenyl Hs), 7.63 (m, 1H, pyrrole H5), 7.81 (m, 2H, phenyl Hs), 9.97 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.6, 112.3, 124.4, 127.4, 129.4, 130.0, 133.4, 135.1, 145.9, 178.9; HRMS calcd for $C_{12}H_{11}NO_3S$ 249.0460, found 249.0466.

**2-Hydroxymethyl-1-[(4-methylphenyl)sulfonyl]pyrrole 3.25**

The *N*-tosylpyrrole **3.24** (82 mg, 0.33 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **3.25** (75 mg, 91%) as a pale pink solid: mp 94 – 96 °C (lit.[12] 97 °C); IR ($CHCl_3$) 3582, 1367, 1175, 1150 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H, Ph*Me*), 2.95 (bs, 1H, O*H*), 4.59 (s, 2H, C*H*$_2$OH), 6.21 – 6.25 (m, 2H, pyrrole H4 & pyrrole H3), 7.25 – 7.29 (m, 3H, pyrrole H5 & phenyl Hs), 7.70 (m, 2H, phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.4, 56.6, 111.7, 114.9, 123.3, 126.5, 130.0, 134.4, 135.8, 145.1; HRMS calcd for $C_{12}H_{13}NO_3S$ 251.0616, found 251.0615.




**2.25b** R = $SO_2Me$ **3.26** R = $SO_2Me$ **3.28** R = $SO_2Me$
**3.25** R = $SO_2C_6H_4Me$ **3.27** R = $SO_2C_6H_4Me$ **3.29** R = $SO_2C_6H_4Me$

**2-Chloromethyl-1-(methanesulfonyl)pyrrole 3.28**

General procedure I was carried out using the *N*-mesylpyrrole **2.25b** (67 mg, 0.38 mmol) and methanesulfonyl chloride (46 μL, 0.58 mmol, 1.5 equiv). The residual oil was dried under high vacuum (oil pump) for 3 h to give the crude **3.28** (65 mg, 87%) as an orange oil which was used in the subsequent reaction without purification: IR ($CHCl_3$) 1369, 1180 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.40 (s, 3H, $SO_2Me$), 4.94 (s, 2H, $CH_2Cl$), 6.27 (m, 1H, pyrrole H4), 6.43 (m, 1H, pyrrole H3), 7.21 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 37.8, 43.2, 111.5, 117.1, 124.2, 130.0; HRMS calcd for $C_6H_8NO_2S$ (M-Cl) 158.0276, found 158.0276.

**2-Chloromethyl-1-[(4-methylphenyl)sulfonyl]pyrrole 3.29**

General procedure I was carried out using the *N*-tosylpyrrole **3.25** (70 mg, 0.28 mmol) and methanesulfonyl chloride (33 μL, 0.42 mmol, 1.5 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **3.29** (62 mg, 83%) as a pale pink solid. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give pale yellow crystals: mp 84 °C; IR ($CHCl_3$) 1371, 1190, 1175, 1150, 1123 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H, Ph*Me*), 4.83 (s, 2H, $CH_2Cl$), 6.24 (m, 1H, pyrrole H4), 6.36 (m, 1H, pyrrole H3), 7.27 – 7.33 (m, 3H, pyrrole H5 & phenyl Hs), 7.78 (m, 2H, phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.5, 37.2, 111.6, 116.9, 124.3, 127.1, 129.8, 130.3, 135.7, 145.2; HRMS calcd for $C_{12}H_{12}ClNO_2S$ 269.0277, found 269.0276. Anal. Calcd for $C_{12}H_{12}ClNO_2S$: C, 53.43; H, 4.48; N, 5.19. Found: C, 53.88; H, 4.65; N, 5.00.

## 8.3.2. $^1$H NMR investigation of the reaction of the pyrrole 2.25b at low temperature

**Reaction of 2-hydroxymethyl-1-(methanesulfonyl)pyrrole 2.25b with methanesulfonyl chloride**

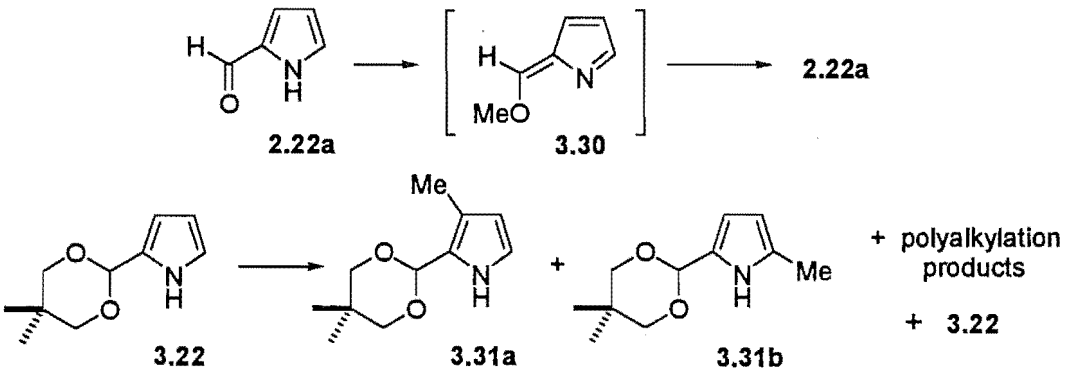
A solution of the *N*-mesylpyrrole **2.25b** (5 mg, 0.03 mmol) in $CDCl_3$ (0.5 mL) containing Hünig's base (7 μL, 0.04 mmol, 1.5 equiv) was cooled to –78 °C in a 5 mm NMR tube under a stream of $N_2$. Methanesulfonyl chloride (3 μL, 0.04 mmol, 1.5 equiv) dissolved in $CDCl_3$ (0.2 mL) was added with mixing. The NMR tube was then inserted into the cooled NMR spectrometer (–20 °C probe temperature) and a $^1$H NMR spectrum of the reaction mixture was recorded (t = 0 min). Spectral analysis showed a mixture of the alcohol **2.25b**, the *O*-mesylate **3.26**, and the chloromethylpyrrole **3.28** (**4 : 5 : 1** by $^1$H NMR). Selected $^1$H NMR ($CDCl_3$, 300 MHz) assigned from the reaction mixture, δ 4.74 (d, $J$ = 5.4 Hz, C$H_2$OH **2.25b**), 4.91 (s, C$H_2$Cl **3.28**), 5.38 (s, C$H_2$OMs **3.26**). The reaction mixture was then kept at –20 °C for 60 min and $^1$H NMR spectra were recorded at regular intervals in order to monitor the progress of the reaction. The reaction mixture was then left at rt and monitored by $^1$H NMR.

**Reaction of 2-hydroxymethyl-1-(methanesulfonyl)pyrrole 2.25b with methanesulfonic anhydride**

A solution of the *N*-mesylpyrrole **2.25b** (5 mg, 0.03 mmol) in $CDCl_3$ (0.5 mL) containing Hünig's base (7 μL, 0.04 mmol, 1.5 equiv) was cooled to –78 °C in a 5 mm NMR tube under a stream of $N_2$. Methanesulfonic anhydride (7 mg, 0.04 mmol, 1.5 equiv) dissolved in $CDCl_3$ (0.2 mL) was added with mixing. The NMR tube was then inserted into the cooled NMR spectrometer (–20 °C probe temperature) and a $^1$H NMR spectrum of the reaction mixture was recorded. Spectral analysis showed that most of the alcohol **2.25b** had been converted into the *O*-mesylated pyrrole **3.26**, with only a trace (~5%) of **2.25b** still remaining. Selected $^1$H NMR ($CDCl_3$, 300 MHz) of **3.26** assigned from the reaction mixture, δ 5.38 (s, 2H, C$H_2$OMs), 6.29 (m, 1H, pyrrole H4), 6.53 (m, 1H, pyrrole H3), 7.23 (m, 1H, pyrrole H5). The reaction mixture was then allowed to warm in 5 °C increments to rt (23 °C) over a period of 30 min. $^1$H NMR spectra at each

5 °C increment were recorded in order to monitor the thermal stability of the *O*-mesylate derivative **3.26**. A $^1$H NMR spectrum of the reaction mixture after 18 h at rt revealed that the *O*-mesylate **3.26** had been completely converted back to the alcohol **2.25b**.

## 8.3.3. Preliminary investigations into pyrrolylmagnesium reactions




**Attempted methylation of 1*H*-pyrrole-2-carboxaldehyde 2.22a**

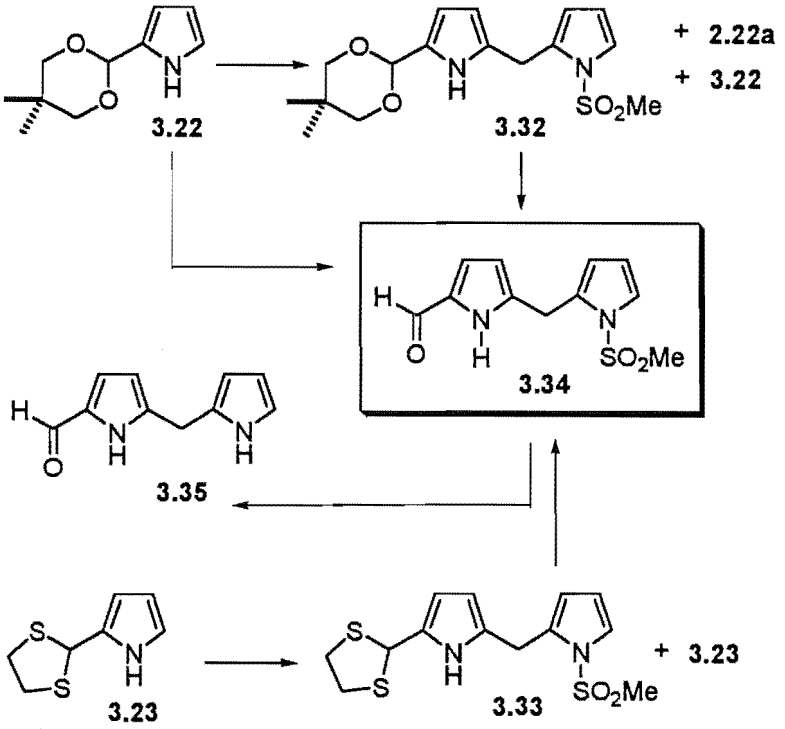
Pyrrole-2-carboxaldehyde **2.22a** (25 mg, 0.26 mmol) was dissolved in THF (5 mL) and the solution was stirred at rt under $N_2$. Methylmagnesium iodide (145 μL of 2.0 M solution in ether, 0.29 mmol, 1.1 equiv) was added and a precipitate immediately formed. After stirring for 30 min, methyl iodide (115 μL, 1.84 mmol, 7 equiv) was added and the mixture was stirred at rt for a further 3 h. The THF was removed under reduced pressure and the residue partitioned between water (10 mL) and dichloromethane (10 mL). The organic phase, together with dichloromethane washings (2x10 mL), was dried and evaporated under reduced pressure to give returned **2.22a** (23 mg).

**Methylation of 2-(5,5-dimethyl-1,3-dioxan-2-yl)-1*H*-pyrrole 3.22**

Methylmagnesium iodide (264 μL of 1.85 M solution in ether, 0.49 mmol, 1.1 equiv) was added to a stirred solution of **3.22** (80 mg, 0.44 mmol) in THF (2.5 mL) at rt under $N_2$, and a precipitate immediately formed. After 30 min, methyl iodide (0.5 mL, 8.03 mmol, 18.1 equiv) was added and the mixture was stirred for 24 h. The THF was removed under reduced pressure and the residue partitioned between water (10 mL) and dichloromethane (10 mL). The organic phase, together with dichloromethane washings (2x10 mL), was dried and evaporated under reduced pressure. Flash chromatography on

silica (ethyl acetate/petroleum ether, 1:3) gave an inseparable mixture of predominantly returned **3.22** and the 2,3- and 2,5-disubstituted pyrroles **3.31a** and **3.31b** (75 mg, **5** : **2** by $^1$H NMR), along with polyalkylation products. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.40 (s, 1H, C*H*O **3.31b**), 5.44 (s, 1H, C*H*O **3.22**), 5.45 (s, 1H, C*H*O **3.31a**).

## 8.3.4. Preparation of dipyrromethanes 3.32 – 3.35




**5-[1-(Methanesulfonyl)pyrrol-2-ylmethyl]-2-(1*H*-pyrrol-2-yl)-1,3-dithiolane 3.33**

Methylmagnesium iodide (0.55 mL of 2.0 M solution in ether, 1.10 mmol, 3.3 equiv) was added to a stirred solution of **3.23** (200 mg, 1.17 mmol, 3.5 equiv) in THF (3 mL) cooled in an ice-sodium chloride bath (–10 °C bath temperature) under $N_2$. The orange suspension was stirred at –10 °C for 30 min and then at rt for a further 30 min. The reaction temperature was lowered to –10 °C and the chloromethylpyrrole **3.28** (65 mg, 0.33 mmol) dissolved in THF (2 mL) was added rapidly. The cooling bath was removed and the reaction mixture was stirred at rt for 2 h. Ether and excess saturated aqueous ammonium chloride were added. The organic phase was separated, washed with saturated aqueous ammonium chloride (10 mL), dried and evaporated under reduced

pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:8) gave recovered **3.23** (134 mg) and **3.33** (55 mg, 50%) as a yellow oil which crystallised on cooling to 0 °C. An analytical sample was obtained by recrystallisation from methanol to give pale orange crystals: mp 108 °C; IR ($CHCl_3$) 3441, 3009, 2932, 1364, 1180 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.57 (s, 3H, $SO_2Me$), 3.36 (m, 4H, $SCH_2$), 4.11 (s, 2H, $CH_2$), 5.73 (s, 1H, C*H*S), 5.81 (m, 1H, pyrrole H4), 6.04 (m, 1H, pyrrole H3), 6.17 (m, 1H, pyrrole H4'), 6.21 (m, 1H, pyrrole H3'), 7.08 (m, 1H, pyrrole H5'), 8.52 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 26.0, 39.8, 42.0, 49.2, 107.3, 108.5, 111.1, 113.6, 122.2, 127.7, 129.1, 132.5; HRMS calcd for $C_{13}H_{16}N_2O_2S_3$ 328.0374, found 328.0372. Anal. Calcd for $C_{13}H_{16}N_2O_2S_3$: C, 47.54; H, 4.91; N, 8.53; S, 29.28. Found: C, 47.28; H, 4.88; N, 8.74; S, 29.37.

**5-[1-(Methanesulfonyl)pyrrol-2-ylmethyl]-1*H*-pyrrole-2-carboxaldehyde 3.34**

**Method A:** A solution of **3.33** (12 mg, 0.04 mmol) in 80% aqueous acetonitrile (1.5 mL) was added to a stirred solution of mercury(II) chloride (20 mg, 0.07 mmol, 2 equiv) and powdered calcium carbonate (11 mg, 0.11 mmol, 3 equiv) in 80% aqueous acetonitrile (1.5 mL). The reaction mixture was stirred at rt for 1 h. Dichloromethane (20 mL) was added and the solution was filtered to remove the mercury precipitate. The organic phase, and subsequent washings of the aqueous phase (2x5 mL), were combined and washed with saturated aqueous sodium hydrogencarbonate (10 mL), saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **3.34** (8 mg, 83%) as a pale orange solid. An analytical sample was obtained by recrystallisation from methanol to give pale orange needles: mp 141 – 142 °C; IR ($CHCl_3$) 3431, 1651, 1367, 1191 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 300 MHz) δ 3.26 (s, 3H, $SO_2Me$), 4.41 (s, 2H, $CH_2$), 6.19 (m, 1H, pyrrole H4'), 6.25 (m, 1H, pyrrole H4), 6.36 (m, 1H, pyrrole H3'), 7.05 (m, 1H, pyrrole H3), 7.25 (m, 1H, pyrrole H5'), 9.56 (s, 1H, C*H*O), 11.10 (bs, 1H, N*H*); $^{13}C$ NMR (acetone-$d_6$, 75 MHz) δ 25.9, 42.1, 110.4, 111.3, 113.9, 121.1, 122.5, 131.9, 133.1, 138.4, 178.3; HRMS calcd for $C_{11}H_{12}N_2O_3S$ 252.0569, found 252.0569. Anal. Calcd for $C_{11}H_{12}N_2O_3S$: C, 52.37; H, 4.79; N, 11.10. Found: C, 52.52; H, 4.86; N, 11.09.

**Method B:** Methylmagnesium iodide (0.52 mL of 2.0 M solution in ether, 1.04 mmol, 3.3 equiv) was added to a stirred solution of **3.22** (201 mg, 1.11 mmol, 3.5 equiv) in THF

(3 mL) under the conditions used above for **3.23** to generate the *N*-magnesio derivative. This was then reacted with the chloromethylpyrrole **3.28** (61 mg, 0.32 mmol), again exactly as for the preparation of **3.33**. After the stirring period of 2 h water (1 mL) was added, followed by 10% aqueous glacial acetic acid (5 mL), and the mixture was stirred for 10 min. Dichloromethane (10 mL) was added and the organic phase, together with subsequent dichloromethane washings (2x10 mL) of the aqueous phase, was washed with saturated aqueous sodium hydrogencarbonate (10 mL), saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave recovered **3.22** (104 mg) and an inseparable mixture of **2.22a** and **3.32** (**1 : 3** by $^1$H NMR) which was used in the next step without further purification. $^1$H NMR ($CDCl_3$, 300 MHz) of **3.32** assigned from the mixture, δ 0.77 (s, 3H, C*Me*$_2$), 1.23 (s, 3H, C*Me*$_2$), 2.58 (s, 3H, SO$_2$*Me*), 3.63 (m, 4H, OC*H*$_2$), 4.13 (s, 2H, C*H*$_2$), 5.39 (s, 1H, C*H*O), 5.91 (m, 1H, pyrrole H), 6.10 (m, 1H, pyrrole H), 6.16 (m, 1H, pyrrole H), 6.20 (m, 1H, pyrrole H), 7.08 (m, 1H, pyrrole H), 8.51 (bs, 1H, N*H*).

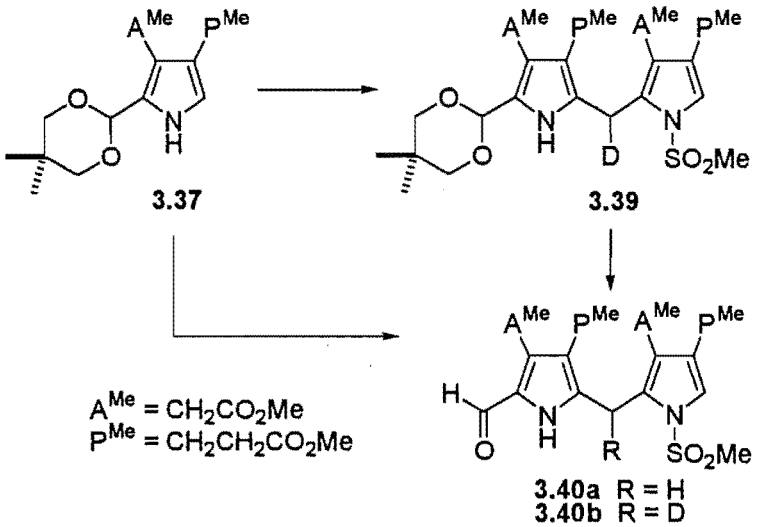
A solution of the crude **3.32** (81 mg, 0.24 mmol) and pyridinium tosylate (6 mg, 0.02 mmol, 0.1 equiv) in 50% aqueous acetone (4 mL) was stirred at reflux for 45 min. Dichloromethane (10 mL) was added and the organic phase, together with subsequent dichloromethane washings (2x5 mL) of the aqueous phase, was washed with saturated aqueous sodium hydrogencarbonate (10 mL), saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) gave **3.34** (48 mg, 60% overall for both steps) as a pale orange solid. Spectral data as recorded above.

**Method C:** The *N*-magnesio salt prepared from **3.22** (96 mg, 0.53 mmol, 3.5 equiv) as detailed above, was reacted with the chloromethylpyrrole **3.28** (29 mg, 0.15 mmol) as described in method B above. The reaction was worked up by the addition of water (1 mL), followed by 10% aqueous hydrochloric acid (5 mL). The mixture was then stirred for 10 min and extracted with dichloromethane as described in method B. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave recovered **2.22a** (37 mg) and **3.34** (24 mg, 63%) as a pale orange solid. Spectral data as recorded above.

**5-(1*H*-Pyrrol-2-ylmethyl)-1*H*-pyrrole-2-carboxaldehyde 3.35**

Aqueous 5 M sodium hydroxide (1 mL) was added to a stirred solution of **3.34** (23 mg, 0.09 mmol) in methanol (4 mL) and the mixture was heated at reflux for 3 h. After cooling to rt, saturated aqueous ammonium chloride (10 mL) was added and the mixture was extracted with dichloromethane (3x10 mL). The combined organic phases were washed with water (2x10 mL), dried and evaporated under reduced pressure. Flash chromatography of the resulting oil on silica (ethyl acetate/petroleum ether, 1:2) gave **3.35** (13 mg, 85%) as a pale orange solid. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give colourless crystals: mp 118 °C (lit.[13] 120 – 121 °C); IR ($CHCl_3$) 3470, 3433, 3292, 3032, 3013, 1645 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 4.02 (s, 2H, C*H*$_2$), 6.03 (m, 1H, pyrrole H4'), 6.11 (m, 1H, pyrrole H3'), 6.16 (m, 1H, pyrrole H4), 6.67 (m, 1H, pyrrole H5'), 6.95 (m, 1H, pyrrole H3), 9.18 (bs, 1H, N*H*'), 9.30 (s, 1H, C*H*O), 11.10 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 26.5, 106.6, 108.3, 110.6, 117.6, 124.6, 127.4, 132.0, 142.2, 178.7; HRMS calcd for $C_{10}H_{10}N_2O$ 174.0793, found 174.0796.

## 8.3.5. Preparation and analysis of dipyrromethanes 3.40a and 3.40b



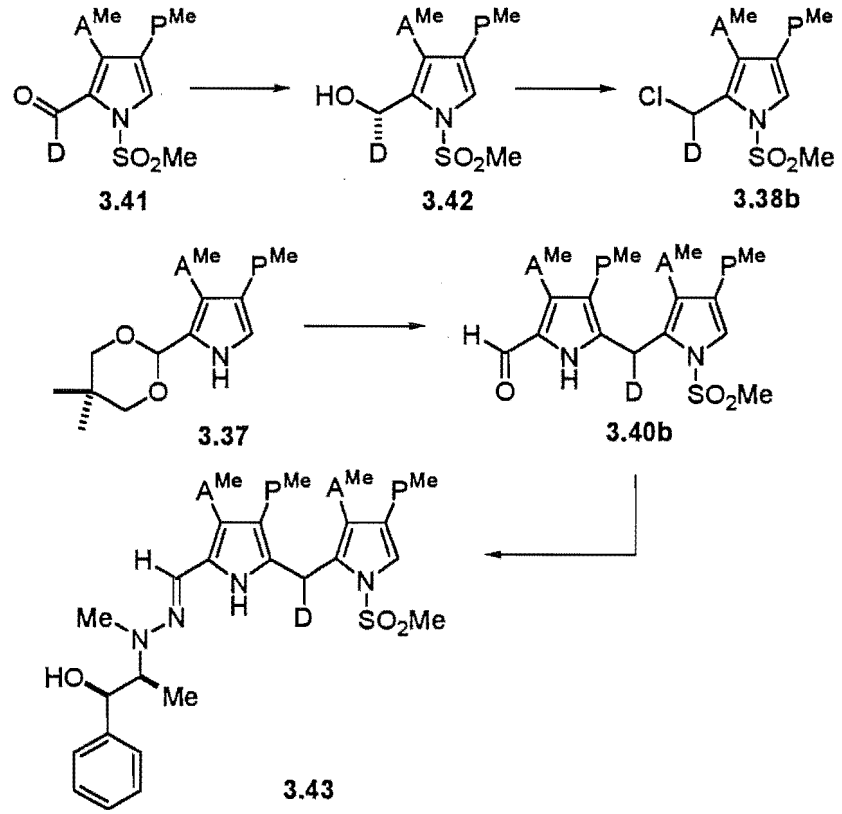
**3,4'-Dimethoxycarbonylethyl-3',4-dimethoxycarbonylmethyl-5-[1-(methanesulfonyl) pyrrol-2-ylmethyl]-1*H*-pyrrole-2-carboxaldehyde 3.40a and the [*methylene-d*$_1$]-analogue 3.40b**

Methylmagnesium iodide (250 μL of 1.2 M solution in ether, 0.36 mmol, 1.2 equiv) was added to a stirred solution of the pyrrole **3.37** (100 mg, 0.30 mmol) in THF (4 mL) and the resultant heterogeneous mixture was stirred at rt under argon for 1 h. The chloromethylpyrrole **3.38a**,[14] in THF (1.5 mL), was added dropwise and the mixture was stirred at rt for 20 h. Water (1 mL) was added, followed by dilute aqueous hydrochloric acid (5 mL), and the mixture was stirred for 10 min. Dichloromethane (20 mL) was added and the organic phase, together with subsequent dichloromethane washings (2x10 mL) of the aqueous phase, were washed with saturated aqueous sodium hydrogencarbonate (10 mL), saturated aqueous brine (10 mL), dried and evaporated. Preparative TLC (ethyl acetate/ether, 1:2) gave the α-formyl derivative of **3.37** (13 mg, 15%) and the *N*-mesyldipyrromethane **3.40a** (141 mg, 75% based on **3.37**) as a pale yellow oil: UV $\lambda_{max}$ 306 nm; IR (film) 2950, 1730, 1640 cm$^{-1}$; $^1$H NMR ($CD_2Cl_2$, 400 MHz) δ 2.41 (s, 3H), 2.52 (m, 2H), 2.57 (m, 2H), 2.69 (m, 2H), 2.87 (m, 2H), 3.57 (s, 2H), 3.64 (s, 3H), 3.66 (s, 3H), 3.67 (s, 3H), 3.81 (s, 3H), 3.77 (s, 2H), 4.15 (s, 2H), 6.91 (s, 1H), 9.51 (s, 1H), 10.10 (bs, 1H); HRMS calcd for $C_{25}H_{32}N_2O_{11}S$ 568.1727, found 568.1731.

The above sequence was repeated using racemic deuterium-labelled chloromethylpyrrole **3.38b**.[14] The *N*-magnesio salt of **3.37**, prepared as described above, was reacted with the chloromethylpyrrole **3.38b** and the resultant solution was stirred at rt for 48 h. Addition of excess water and aqueous acetic acid, followed by preparative TLC (ethyl acetate/ether, 1:2) gave recovered starting material **3.37** (26%) and the labelled dipyrromethane **3.39** (70%) as a labile pale yellow oil: $^1$H NMR ($CD_2Cl_2$, 400 MHz) δ 1.24 (s, 3H), 1.37 (s, 3H), 2.33 (s, 3H), 2.43 (m, 2H), 2.56 (m, 2H), 2.67 (m, 2H), 2.78 (m, 2H), 3.47 (s, 2H), 3.55 (s, 2H), 3.62 (s, 3H), 3.64 (s, 3H), 3.66 (s, 3H), 3.75 (s, 3H), 4.04 (s, 1H), 5.34 (s, 1H), 6.90 (s, 1H), 9.36 (bs, 1H); $^{13}$C NMR ($CD_2Cl_2$, 63 MHz) δ 19.5, 20.8, 21.5 (t, CHD), 22.0, 23.0, 29.9, 30.1, 33.8, 35.7, 41.7, 51.8, 51.9, 52.0, 52.5, 71.7, 77.8, 96.3, 113.1, 118.9, 119.5, 123.8, 124.7, 126.4, 130.2, 172.9, 173.0, 173.8, 173.9.

The preceding dipyrromethane **3.39** in dichloromethane was shaken with dilute aqueous hydrochloric acid for 10 min. The organic phase was separated, dried and evaporated to give the labelled dipyrromethane **3.40b** as an oil: $^1$H NMR ($CD_2Cl_2$, 400 MHz) δ 4.13 (s, 1H) otherwise identical to the spectrum of **3.40a**; $^{13}$C NMR ($CDCl_3$, 63 MHz) δ 18.7, 20.4, 21.4 (t, CHD), 29.5, 30.8, 33.4, 34.7, 41.9, 51.6, 51.8, 52.2, 53.1, 119.1, 119.9, 122.7, 124.8, 128.0, 129.1, 133.6, 171.3, 172.9, 173.0, 173.2, 177.3.

**Attempted formation of chiral deuterium-labelled *N*-mesyl dipyrromethane 3.40b and subsequent formation of the labelled hydrazone derivative 3.43**



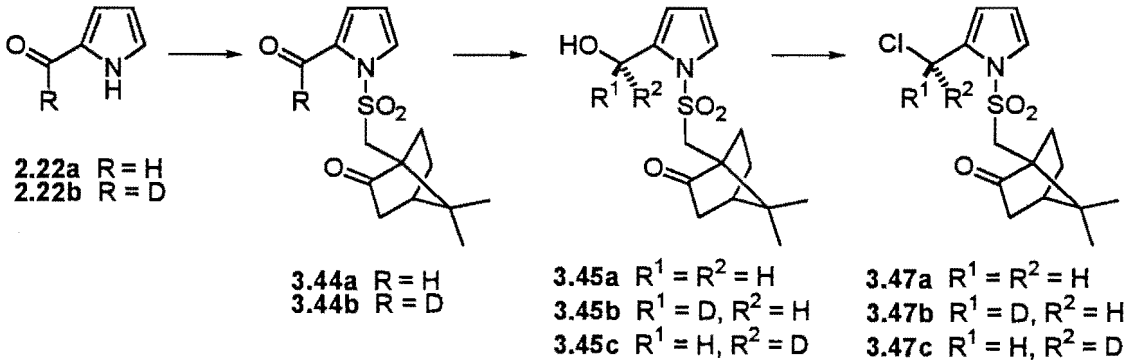

A solution of 9-BBN (4.05 mL of 0.5 M solution in THF, 1.12 equiv) and (1*R*)-(+)-α-pinene (360 μL, 1.25 equiv) was refluxed under $N_2$ for 2 h. The solution was cooled to rt and the deuterium-labelled formylpyrrole **3.41**[14] (600mg, 1.81 mmol) in THF (6.5 mL) was added. After 2 h at rt acetaldehyde (8 drops) was added. The THF was removed under reduced pressure, and the α-pinene was then removed on a vacuum pump for 1 h. Dichloromethane (30 mL) was added and the solution was cooled to 0 °C. 2-Aminoethanol (180 μL) was added and the resultant precipitate was removed by

filtration. The filtrate was washed with water, dried and evaporated under reduced pressure. Preparative TLC (ether/hexane, 9:1) gave the deuterated hydroxymethylpyrrole **3.42** (507 mg, 84%) which crystallised on standing: $^1$H NMR ($CDCl_3$, 400 MHz) 2.56 (m, 2H), 2.70 (m, 2H), 3.10 (d, $J$ = 6.6 Hz, 1H), 3.35 (s, 3H), 3.49 (s, 2H), 3.67 (s, 3H), 3.73 (s, 3H), 4.74 (d, $J$ = 6.6 Hz, 1H), 6.94 (s, 1H); HRMS calcd for $C_{13}H_{18}DNO_7S$ 334.0945, found 334.0948.

The deuterium-labelled chloromethylpyrrole **3.38b** was prepared from this optically active sample of *N*-mesylhydroxymethylpyrrole **3.42** by the methodology described by Abell *et al.*[14] Subsequent reaction of this chloromethylpyrrole **3.38b** with the *N*-magnesio salt of **3.37**, prepared as described above, followed by work up as described for **3.40a**, gave the α-formyl derivative of **3.37** (28%) and the labelled α-formyldipyrromethane **3.40b** (66%). Spectral data as recorded above.

A solution of **3.40b** and *N*-amino-*l*-ephedrine[15] in benzene (2.5 mL) containing activated 4 Å molecular sieves was heated at reflux for 2 – 5 h under $N_2$ and then left at rt for 18 h. The sieves were removed by filtration and the filtrate was evaporated under reduced pressure. Preparative TLC (ether) gave the deuterium-labelled hydrazone **3.43**: $^1$H NMR ($CD_6D_6$, 400 MHz) δ 1.18 (d, $J$ = 6.7 Hz, 3H, CH*Me*), 2.09 (s, 3H, $SO_2$*Me*), 2.32 (m, 2H, $CH_2CH_2CO_2$), 2.39 (s, 3H, N*Me*), 2.51 (t, $J$ = 7.5 Hz, 2H, $CH_2CH_2CO_2$), 2.66 (t, $J$ = 7.5 Hz, 2H, $CH_2CH_2CO_2$), 3.01 (s, 2H, $CH_2CO_2$), 3.19 (m, 3H, $CH_2CH_2CO_2$ & C*H*Me), 3.26 (s, 3H, $CO_2$*Me*), 3.29 (s, 3H, $CO_2$*Me*), 3.36 (s, 3H, $CO_2$*Me*), 3.53 (s, 3H, $CO_2$*Me*), 3.58 (s, 2H, $CH_2CO_2$), 3.79 (bs, 1H, O*H*), 3.89 (s, 0.5H, C*H*D), 3.97 (s, 0.5H, C*H*D), 5.23 (d, $J$ = 2.2 Hz, 1H, C*H*OH), 6.92 (s, 1H, pyrrole H), 7.10 – 7.40 (m, 6H, C*H*N & phenyl Hs), 10.05 (bs, 1H, N*H*); HRMS calcd for $C_{35}H_{45}DN_4O_{11}S$ 731.2947, found 731.2946.

## 8.3.6. Preparation of unlabelled and [*methylene-d*$_1$]-labelled 2-chloromethyl-1-camphorsulfonylpyrroles 3.47a-c




**(1*S*,4*R*)-1-(10-Camphorsulfonyl)pyrrole-2-carboxaldehyde 3.44a and [*formyl-d*]-(1*S*,4*R*)-1-(10-camphorsulfonyl)pyrrole-2-carboxaldehyde 3.44b**

General procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (20 mg, 0.21 mmol) and (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride[16] (63 mg, 0.25 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **3.44a** (62 mg, 95%) as a colourless oil which solidified at 0 °C. An analytical sample was obtained by recrystallisation from petroleum ether to give a white solid: mp 97 °C; IR ($CHCl_3$) 1747, 1678, 1377, 1171 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 3H, camph-Me), 1.20 (s, 3H, camph-Me), 1.48 (m, 1H, camph-CH), 1.78 (m, 1H, camph-CH), 1.92 – 2.17 (m, 3H, camph-CH), 2.36 – 2.55 (m, 2H, camph-CH), 3.94 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 4.08 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 6.41 (m, 1H, pyrrole H4), 7.22 (m, 1H, pyrrole H3), 7.61 (m, 1H, pyrrole H5), 9.72 (s, 1H, C*H*O); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 19.6, 19.7, 25.2, 27.0, 42.5, 42.7, 48.3, 52.6, 58.9, 111.3, 128.9, 130.7, 133.1, 178.2, 213.9; HRMS calcd for $C_{15}H_{19}NO_4S$ 309.1035, found 309.1037.

The reaction was repeated using [*formyl-d*]-pyrrole-2-carboxaldehyde **2.22b** (20 mg, 0.21 mmol) to give **3.44b** (64 mg, 99%): $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 19.4, 19.5, 25.0, 26.7, 42.2, 42.4, 48.1, 52.4, 58.7, 111.2, 128.7, 130.5, 132.8, 177.8 (t, $J$ = 28.2 Hz), 213.7; HRMS calcd for $C_{15}H_{18}DNO_4S$ 310.1098, found 310.1094.

**(1*S*,4*R*)-2-Hydroxymethyl-1-(10-camphorsulfonyl)pyrrole 3.45a**

The *N*-camphorsulfonylpyrrole **3.44a** (27 mg, 0.09 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **3.45a** (24 mg, 88%) as a colourless oil: IR ($CHCl_3$) 3514, 1746, 1369, 1175 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.87 (s, 3H, camph-Me), 1.10 (s, 3H, camph-Me), 1.50 (m, 1H, camph-CH), 1.85 (m, 1H, camph-CH), 1.95 – 2.17 (m, 3H, camph-CH), 2.37 – 2.50 (m, 2H, camph-CH), 3.29 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 3.63 (bs, 1H, O*H*), 3.83 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 4.73 (ABq, $J$ = 13.2 Hz, 1H, $CH_2OH$), 4.88 (ABq, $J$ = 13.2 Hz, 1H, $CH_2OH$), 6.23 (m, 1H, pyrrole H4), 6.29 (m, 1H, pyrrole H3), 7.15 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 19.5, 19.6, 25.3, 26.8, 42.5, 42.8, 48.3, 52.8, 56.4, 58.7, 111.0, 115.1, 123.1, 134.2, 214.7; HRMS calcd for $C_{15}H_{21}NO_4S$ 311.1191, found 311.1193.

**(1*S*,4*R*)-2-Chloromethyl-1-(10-camphorsulfonyl)pyrrole 3.47a**

A solution of the *N*-camphorsulfonylpyrrole **3.45a** (7 mg, 0.02 mmol) in $CDCl_3$ (150 μL) containing Hünig's base (11 μL, 0.06 mmol, 3 equiv) in a 3 mm NMR tube was cooled in an ice bath under a stream of $N_2$. Methanesulfonyl chloride (5 μL, 0.06 mmol, 3 equiv) was added with mixing and the progress of the reaction was then monitored by $^1H$ NMR. When no more alcohol **3.45a** could be observed by $^1H$ NMR spectroscopy, the reaction was poured into dichloromethane (10 mL), washed with ice-cold water (10 mL), cold 10% aqueous hydrochloric acid (10 mL), and saturated aqueous sodium hydrogencarbonate (10 mL). The organic phase was then dried and evaporated under reduced pressure. The residual oil was dried under high vacuum (oil pump) for 3 h to give the crude **3.47a** (7 mg) as a yellow oil. $^1H$ NMR showed the compound to be *ca.* 90% pure, with 10% non-pyrrole material: IR ($CHCl_3$) 1747, 1371, 1177 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.90 (s, 3H, camph-Me), 1.20 (s, 3H, camph-Me), 1.48 (m, 1H, camph-CH), 1.79 (m, 1H, camph-CH), 1.94 – 2.15 (m, 3H, camph-CH), 2.37 – 2.62 (m, 2H, camph-CH), 3.56 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 3.82 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 4.86 (ABq, $J$ = 12.7 Hz, 1H, $CH_2Cl$), 5.07 (ABq, $J$ = 12.7 Hz, 1H, $CH_2Cl$), 6.27 (m, 1H, pyrrole H4), 6.43 (m, 1H, pyrrole H3), 7.25 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 19.7, 19.9, 25.2, 27.0, 38.0, 42.5, 42.7, 48.1, 52.9, 59.0, 111.3, 117.1, 124.5, 129.8, 213.8; HRMS calcd for $C_{15}H_{20}ClNO_3S$ 329.0852, found 329.0851.

**[*methylene-d*$_1$]-(1*S*,4*R*)-2-Chloromethyl-1-(10-camphorsulfonyl)pyrrole 3.47b and 3.47c**

***S*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-camphorsulfonylpyrrole **3.44b** (12 mg, 0.04 mmol) and *S*-Alpine borane® (86 μL of 0.5 M solution in THF, 0.04 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave a mixture of **3.45b** and **3.45c** (9 mg, 70%, 19:1) as a colourless oil: $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.87 (s, 3H, camph-Me), 1.11 (s, 3H, camph-Me), 1.51 (m, 1H, camph-CH), 1.86 (m, 1H, camph-CH), 1.95 – 2.17 (m, 3H, camph-CH), 2.37 – 2.50 (m, 2H, camph-CH), 3.28 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 3.58 (bs, 1H, O*H*), 3.84 (ABq, $J$ = 14.7 Hz, 1H, $SO_2CH_2$), 4.72 (s, 0.95H, C*H*DOH **3.45b**), 4.87 (s, 0.05H, C*H*DOH **3.45c**), 6.23 (m, 1H, pyrrole H4), 6.30 (m, 1H, pyrrole H3), 7.15 (m, 1H, pyrrole H5); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 19.6, 19.7, 25.4, 26.9, 42.5, 42.9, 48.3, 52.9, 56.3 (t, $J$ = 21.9 Hz), 58.7, 111.1, 115.1, 123.2, 134.4, 214.7; HRMS calcd for $C_{15}H_{20}DNO_4S$ 312.1254, found 312.1260.

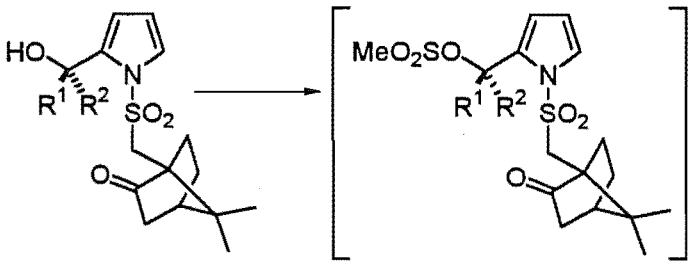
The preceding sample of **3.45b** and **3.45c** (5 mg, 0.02 mmol) in $CDCl_3$ (150 μL) in a 3 mm NMR tube was treated with methanesulfonyl chloride (4 μL, 0.05 mmol, 3 equiv) under the conditions used for the preparation of **3.47a**. $^1$H NMR spectral analysis of the crude reaction mixture showed a mixture of **3.47b** and **3.47c** (~2:3). Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 4.86 (s, 0.6H, C*H*DCl **3.47c**), 5.05 (s, 0.4H, C*H*DCl **3.47b**). Subsequent work up of the reaction mixture by the method described for **3.47a** gave a crude mixture of **3.47b** and **3.47c** (8 mg, ~1:1 by $^1$H NMR) as a pale yellow oil. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 4.86 (s, 0.5H, C*H*DCl **3.47c**), 5.05 (s, 0.5H, C*H*DCl **3.47b**). HRMS calcd for $C_{15}H_{19}DClNO_3S$ 330.0915, found 330.0918.

***R*-Alpine borane® sequence:** General procedure F was carried out using the deuterated *N*-camphorsulfonylpyrrole **3.44b** (12 mg, 0.04 mmol) and *R*-Alpine borane® (83 μL of 0.5 M solution in THF, 0.04 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave a mixture of **3.45b** and **3.45c** (9 mg, 76%, 1:19) as a colourless oil: Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 4.72 (s, 0.05H, C*H*DOH **3.45b**), 4.87 (s, 0.95H, C*H*DOH **3.45c**).

The preceding sample of **3.45b** and **3.45c** (5 mg, 0.02 mmol) in $CDCl_3$ (150 μL) in a 3 mm NMR tube was treated with methanesulfonyl chloride (4 μL, 0.05 mmol, 3 equiv) under the conditions used for the preparation of **3.47a**. $^1$H NMR spectral analysis

of the crude reaction mixture showed a mixture of **3.47b** and **3.47c** (~3:2). Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 4.86 (s, 0.4H, C*H*DCl **3.47c**), 5.05 (s, 0.6H, C*H*DCl **3.47b**). Subsequent work up of the reaction mixture by the method described for **3.47a** gave a crude mixture of **3.47b** and **3.47c** (6 mg, ~1:1) as a pale yellow oil. Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 4.86 (s, 0.5H, C*H*DCl **3.47c**), 5.05 (s, 0.5H, C*H*DCl **3.47b**).

## 8.3.7. $^1$H NMR investigation of the reaction of [*methylene-d*$_1$]-labelled pyrroles 3.45b and 3.45c at low temperature




**3.45b** $R^1$ = D, $R^2$ = H
**3.45c** $R^1$ = H, $R^2$ = D

**3.46b** $R^1$ = D, $R^2$ = H
**3.46c** $R^1$ = H, $R^2$ = D

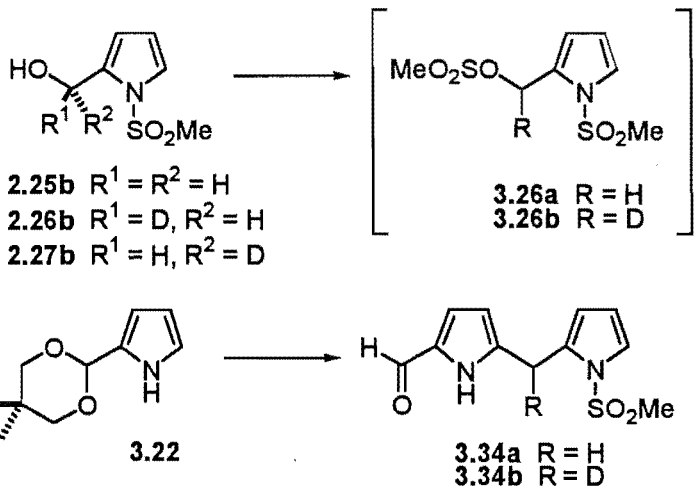
**Reaction of [*methylene-d*$_1$]-(1*S*,4*R*)-2-hydroxymethyl-1-(10-camphorsulfonyl)pyrrole 3.45b and 3.45c with methanesulfonic anhydride**

A sample of **3.45b** and **3.45c** (4 mg, 0.01 mmol, 19:1), obtained from the *S*-Alpine borane® sequence described above, in $CDCl_3$ (0.5 mL) containing Hünig's base (3 μL, 0.02 mmol, 1.5 equiv) was cooled to –78 °C in a 5 mm NMR tube under a stream of $N_2$. Methanesulfonic anhydride (3 mg, 0.02 mmol, 1.5 equiv) dissolved in $CDCl_3$ (0.2 mL) was added with mixing. The NMR tube was then inserted into the cooled NMR spectrometer (–20 °C probe temperature) and a $^1$H NMR spectrum of the reaction mixture was recorded (t = 0 min). Spectral analysis of the crude reaction mixture showed a mixture of **3.46b** and **3.46c** (~4:1). Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.39 (s, 0.2H, C*H*DOMs **3.46c**), 5.45 (s, 0.8H, C*H*DOMs **3.46b**). The reaction mixture was then kept at –20 °C and $^1$H NMR spectra were recorded at regular intervals in order to monitor the progress of the reaction. A $^1$H NMR spectrum of the reaction mixture after 30 min showed an equal mixture of the *O*-mesylated pyrroles **3.46b** and **3.46c** (~1:1). Selected

$^1$H NMR ($CDCl_3$, 300 MHz) δ 5.39 (s, 0.5H, C*H*DOMs **3.46c**), 5.45 (s, 0.5H, C*H*DOMs **3.46b**).

A sample of **3.45b** and **3.45c** (3 mg, 0.01 mmol, 1:19), obtained from the *R*-Alpine borane® sequence above, was treated with methanesulfonic anhydride (3 mg, 0.02 mmol, 1.5 equiv) as described in above. $^1$H NMR spectral analysis of the crude reaction mixture showed a mixture of **3.46b** and **3.46c** (~1:4). Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.39 (s, 0.8H, C*H*DOMs **3.46c**), 5.45 (s, 0.2H, C*H*DOMs **3.46b**). The reaction mixture was then kept at –20 °C and $^1$H NMR spectra were recorded at regular intervals in order to monitor the progress of the reaction. A $^1$H NMR spectrum of the reaction mixture after 90 min showed an equal mixture of the *O*-mesylated pyrroles **3.46b** and **3.46c** (~1:1). Selected $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.39 (s, 0.5H, C*H*DOMs **3.46c**), 5.45 (s, 0.5H, C*H*DOMs **3.46b**).

## 8.3.8. Preparation and attempted analysis of unlabelled and [*methylene-d*$_1$]-labelled dipyrromethanes 3.34a and 3.34b



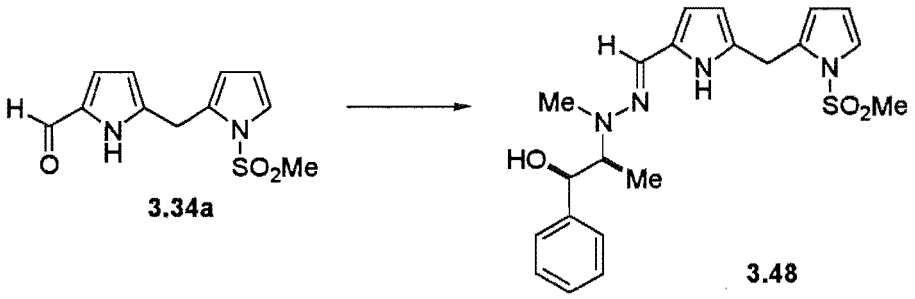

**5-[1-(Methanesulfonyl)pyrrol-2-ylmethyl]-1*H*-pyrrole-2-carboxaldehyde 3.34a and [*methylene-d*$_1$]-5-[1-(methanesulfonyl)pyrrol-2-ylmethyl]-1*H*-pyrrole-2-carboxaldehyde 3.34b**

Methanesulfonic anhydride (45 mg, 0.26 mmol, 1.5 equiv) was added to a stirred solution of **2.25b** (30 mg, 0.17 mmol) in dichloromethane (1 mL) containing Hünig's

base (45 μL, 0.26 mmol, 1.5 equiv) cooled to –40 °C under $N_2$. The mixture was stirred at –40 °C for 2 min and the generated *O*-mesylpyrrole **3.26a** was rapidly transferred by cannular into a solution of the *N*-magnesio salt of **3.22** (109 mg, 0.60 mmol, 3.5 equiv), prepared as described in method B of Section 8.3.3, which was cooled to –40 °C under $N_2$. The resulting mixture was stirred at –40 °C for 2 h and then at rt for 18 h. The preparation was worked up by the addition of water (1 mL) followed by 10% aqueous hydrochloric acid (5 mL). The mixture was then stirred for 10 min and extracted with dichloromethane as described in method B. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave recovered **2.22a** (34 mg) and **3.34a** (23 mg, 53%) as a pale orange solid. Spectral data as recorded above.

The above sequence was repeated using the deuterium-labelled *O*-mesylpyrrole **3.26b**. Thus, the deuterated *O*-mesylpyrrole **3.26b**, prepared as described above from a mixture of the deuterated hydroxymethylpyrroles **2.26b** and **2.27b** (39 mg, 0.22 mmol, ~1:15), was reacted with the *N*-magnesio salt of **3.22** (140 mg, 0.77 mmol, 3.5 equiv), prepared as detailed above. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave recovered **2.22a** (59 mg) and **3.34b** (30 mg, 54%) as a pale orange solid: $^1$H NMR (acetone-$d_6$, 300 MHz) δ 3.25 (s, 3H, $SO_2Me$), 4.39 (s, 1H, C*H*D), 6.19 (m, 1H, pyrrole H4'), 6.25 (m, 1H, pyrrole H4), 6.36 (m, 1H, pyrrole H3'), 7.06 (m, 1H, pyrrole H3), 7.25 (m, 1H, pyrrole H5'), 9.55 (s, 1H, C*H*O), 11.16 (bs, 1H, N*H*); $^{13}$C NMR (acetone-$d_6$, 75 MHz) δ 25.6 (t, $J$ = 20.3 Hz), 42.0, 110.4, 111.3, 113.9, 121.1, 122.4, 131.8, 133.1, 138.4, 178.3; HRMS calcd for $C_{11}H_{11}DN_2O_3S$ 253.0631, found 253.0629.

**Formation of the hydrazone derivative of α-formyldipyrromethane 3.34a**




A solution of **3.34a** (19 mg, 0.08 mmol) and *N*-amino-*l*-ephedrine (41 mg, 0.23 mmol, 3 equiv) in benzene (3 mL) containing activated 4 Å molecular sieves was heated at reflux for 4 h under $N_2$. After cooling to rt, the sieves were removed by filtration and the filtrate evaporated under reduced pressure. Flash chromatography of the resulting oil on silica (ethyl acetate/petroleum ether, 1:4) gave **3.48** (19 mg, 62%) as an unstable colourless oil which rapidly decomposed on exposure to air and in the NMR solvent. $^1$H NMR showed the compound to be *ca.* 95% pure: IR ($CHCl_3$) 3454, 3342, 1724, 1366, 1180 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 1.05 (d, $J$ = 6.8 Hz, 3H, CH*Me*), 2.75 (s, 3H, $SO_2$*Me*), 2.84 (s, 3H, N*Me*), 3.38 (m, 1H, C*H*Me), 4.17 (s, 2H, $CH_2$), 4.29 (bs, 1H, O*H*), 5.21 (m, 1H, C*H*OH), 5.97 (m, 1H, pyrrole H4), 6.14 – 6.24 (m, 3H, pyrrole H3 & pyrrole H4' & pyrrole H3'), 7.10 – 7.41 (m, 7H, pyrrole H5' & C*H*N & phenyl Hs), 8.69 (bs, 1H, N*H*); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.5, 26.1, 37.7, 42.3, 67.3, 76.6, 108.5, 109.6, 111.4, 113.8, 122.3, 126.1, 126.7, 127.1, 128.1, 129.2, 129.9, 132.3, 142.7; HRMS calcd for $C_{21}H_{26}N_4O_3S$ 414.1726, found 414.1726.

**Attempted *N*-substitution of dipyrromethanes 3.34a and 3.35 with (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride**

Attempts to *N*-substitute **3.34a** (typically 18 mg, 0.07 mmol) with (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride (1.4 equiv) by general procedure B proved unsuccessful, giving predominantly returned starting material **3.34a**. Similarly, an attempt to *N*-substitute **3.35** (7 mg, 0.04 mmol) with (1*S*,4*R*)-(+)-10-camphorsulfonyl chloride (2.4 equiv) by the same methodology also proved unsuccessful.

**Attempted analysis of 3.34b by $^2$H NMR in a chiral liquid crystalline solvent**

The deuterium-labelled dipyrromethane **3.34b** (10 mg) was dissolved in $CH_2Cl_2$ (500 μL) and added to poly-γ-benzyl-*L*-glutamate (80 mg, 16% *w/v*), which had been weighed directly into a 5 mm NMR tube. Once all the compounds had dissolved in the NMR tube, the sample was vortexed until an optically homogeneous birefringent phase was obtained. The NMR tube was then inserted into the NMR spectrometer and a $^2$H NMR spectrum of the mixture recorded. $^2$H NMR ($CH_2Cl_2$, 46 MHz) referenced to $CDCl_3$ [7.25 ppm], δ 4.20 (s, 1D, CH*D*).

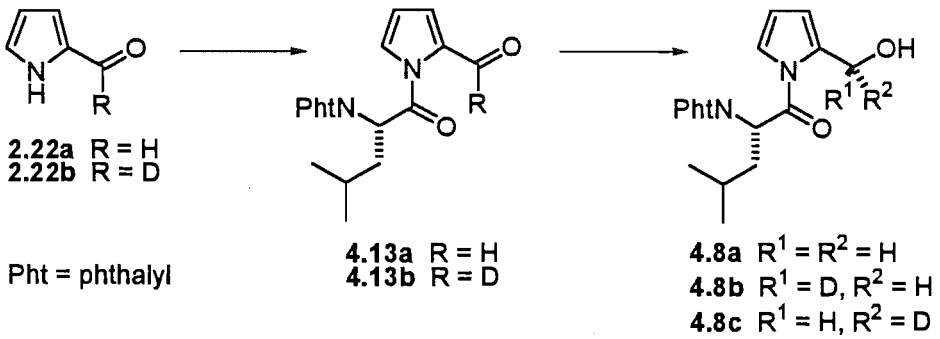
**Crystallographic structure determination for compound 3.29 by X-ray analysis**

$C_{12}H_{12}ClNO_2S$, M 269.74, mp 84 °C, crystal dimensions 1.02 x 0.32 x 0.25 mm, monoclinic, *a* 7.645(2) Å, *b* 15.689(5) Å, *c* 10.543(3) Å, *β* 105.20(2)°, *V* = 1220.3(6) Å$^3$, space group $P2_1/n$, $Z = 4$, F(000) = 560, $D_{calc}$ = 1.468 mg/m$^3$, absorption coefficient 0.472 mm$^{-1}$, *θ* range for data collection 2.39 – 22.50, index ranges $-8 \le h \le 0$, $0 \le k \le 16$, $-10 \le l \le 11$, maximum and minimum transmissions 0.3008 and 0.3345, data/restraints/ parameters 1593/0/155, goodness of fit on $F^2$ was 1.097, final *R* indices [$I>2\sigma(I)$] $R_1$ = 0.0330, $wR_2$ = 0.0849, *R* indices (all data) $R_1$ = 0.0378, $wR_2$ = 0.0883, largest difference peak and hole 0.284 and –0.207 eÅ$^{-3}$.

The unit cell parameters were obtained by least-squares refinement of the setting angles of 19 reflections with $4.25° \le 2\theta \le 16.5°$ from a Siemens four circle diffractometer. A unique data set was measured at 293(2) K within $2\theta_{max} = 45°$ limit (*ω* scans). Of the 1611 reflections obtained, 1593 were unique ($R_{int}$ = 0.0051) and were used in the full-matrix least-squares refinement.[17] The intensities of 3 standard reflections, measured every 97 reflections throughout the data collection, showed only 3.97% decay. The structure was solved by direct methods.[18] Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.[19] Full details of the X-ray structural determination of **3.29** have been deposited with the Cambridge Crystallographic Data Centre (CCDC).

# 8.4 Experimental Work Described in Chapter Four

## 8.4.1. Preparation of unlabelled and [*methylene-d*$_1$]-labelled hydroxymethylpyrroles 4.8a-c




***N*-Phthalyl-*L*-leucine acid fluoride and *N*-phthalyl-*DL*-leucine acid fluoride**

To a stirred solution of *N*-phthalyl-*L*-leucine (60 mg, 0.23 mmol) in dichloromethane (3 mL) was added diethylaminosulfur trifluoride (37 μL, 0.28 mmol, 1.2 equiv) at rt under $N_2$. After 10 min the orange mixture was washed with ice water. The organic layer was then separated, dried and evaporated under reduced pressure. The residual oil was dried under high vacuum (oil pump) for 2 h to give the crude acid fluoride as a yellow oil which was used in the next step without purification: IR ($CHCl_3$) 1850, 1778, 1726 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.95 (d, $J$ = 6.8 Hz, 3H, CH*Me*$_2$), 0.97 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 1.52 (m, 1H, C*H*Me$_2$), 1.95 (m, 1H, C*H*$_2$), 2.37 (m, 1H, C*H*$_2$), 5.14 (m, 1H, α*H*), 7.79 (m, 2H, Pht Hs), 7.89 (m, 2H, Pht Hs).

The reaction was repeated using *N*-phthalyl-*DL*-leucine (60 mg, 0.23 mmol) to give the crude racemic acid fluoride as a yellow oil which was used in the next step without purification. Spectral data as recorded above.

**1-(*N*-Phthalyl-*L*-leucinyl)pyrrole-2-carboxaldehyde 4.13a and [*formyl-d*]-1-(*N*-phthalyl-*L*-leucinyl)pyrrole-2-carboxaldehyde 4.13b**

To a stirred suspension of sodium hydride (7 mg, 0.23 mmol in 80% suspension in oil, washed twice with petroleum ether, 1.1 equiv) in THF (5 mL) under $N_2$ was added pyrrole-2-carboxaldehyde **2.22a** (20 mg, 0.21 mmol) dissolved in THF (1 mL). To the ice-cooled mixture was added a solution of *N*-phthalyl-*L*-leucine acid fluoride (61 mg, 0.23 mmol, 1.1 equiv) in THF (4 mL) over 15 min. The mixture was left to stir at 0 °C for 30 min, and at rt overnight. The mixture was then diluted with ethyl acetate (10 mL), washed with 2 M aqueous potassium hydrogensulfate (10 mL), water (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **4.13a** (62 mg, 87%) as a pale yellow oil: IR ($CHCl_3$) 1776, 1736, 1719, 1666 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.98 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 1.04 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 1.60 (m, 1H, C*H*Me$_2$), 2.01 (m, 1H, C*H*$_2$), 2.42 (m, 1H, C*H*$_2$), 5.68 (dd, $J$ = 10.7 & 4.4 Hz, 1H, α*H*), 6.31 (m, 1H, pyrrole H4), 7.17 (m, 1H, pyrrole H3), 7.30 (m, 1H, pyrrole H5), 7.78 (m, 2H, Pht Hs), 7.86 (m, 2H, Pht Hs), 10.14 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.4, 23.0, 24.9, 37.5, 51.5, 113.2, 122.6, 123.8, 125.5, 131.2, 134.6, 135.3, 167.3, 168.7, 181.7.

The reaction was repeated using [*formyl-d*]-pyrrole-2-carboxaldehyde **2.22b** (25 mg, 0.26 mmol) and *N*-phthalyl-*L*-leucine acid fluoride (75 mg, 0.29 mmol, 1.1 equiv) to give **4.13b** (81 mg, 91%): $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.2, 22.9, 24.7, 37.4, 51.4, 113.1, 122.4, 123.6, 125.5, 131.0, 134.5, 135.3, 167.2, 168.6, 181.2 (t, $J$ = 30.3 Hz).

**2-Hydroxymethyl-1-(*N*-phthalyl-*L*-leucinyl)pyrrole 4.8a**

The *N*-leucinylpyrrole **4.13a** (30 mg, 0.09 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **4.8a** (27 mg, 88%) as a colourless oil which rapidly changed to a red colour when exposed to air: IR ($CHCl_3$) 3551, 1776, 1720, 1383 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.98 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 1.05 (d, $J$ = 6.8 Hz, 3H, CH*Me*$_2$), 1.60 (m, 1H, C*H*Me$_2$), 2.00 (m, 1H, C*H*$_2$), 2.46 (m, 1H, C*H*$_2$), 3.52 (t, $J$ = 6.8 Hz, 1H, O*H*), 4.62 (d, $J$ = 6.8 Hz, 2H, C*H*$_2$OH), 5.54 (m, 1H, α*H*), 6.17 (m, 1H, pyrrole H4), 6.23 (m, 1H, pyrrole H3), 7.06 (m, 1H, pyrrole H5), 7.76 (m, 2H, Pht Hs), 7.87 (m, 2H, Pht Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.4, 23.0, 25.0, 37.6, 51.3, 57.5, 113.0,

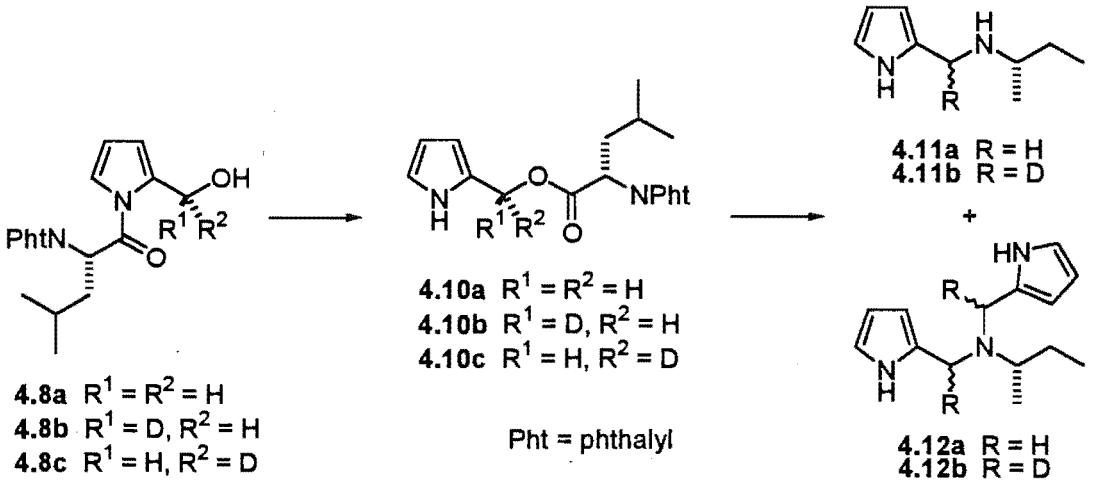
114.8, 120.1, 123.7, 131.3, 134.5, 135.9, 167.4, 169.3; HRMS calcd for $C_{19}H_{20}N_2O_4$ 340.1423, found 340.1425.

**[*methlyene-d*$_1$]-2-Hydroxymethyl-1-(*N*-phthalyl-*L*-leucinyl)pyrrole 4.8b and 4.8c**

***S*-Alpine borane® sequence:** General procedure F was carried out using a sample of the deuterated *N*-leucinylpyrrole **4.13b** (31 mg, 0.09 mmol) and *S*-Alpine borane® (198 μL of 0.5 M solution in THF, 0.10 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.8b** and **4.8c** (27 mg, 88%, >9:1 by $^1$H NMR) as a colourless oil which rapidly changed to a red colour when exposed to air: $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.98 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 1.05 (d, $J$ = 6.8 Hz, 3H, CH*Me*$_2$), 1.60 (m, 1H, C*H*Me$_2$), 2.00 (m, 1H, C*H*$_2$), 2.46 (m, 1H, C*H*$_2$), 3.52 (bs, 1H, O*H*), 4.61 (s, 1H, C*H*DOH), 5.54 (m, 1H, α*H*), 6.17 (m, 1H, pyrrole H4), 6.23 (m, 1H, pyrrole H3), 7.06 (m, 1H, pyrrole H5), 7.76 (m, 2H, Pht Hs), 7.87 (m, 2H, Pht Hs); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 21.4, 23.0, 25.0, 37.6, 51.3, 57.2 (t, $J$ = 21.4 Hz), 113.0, 114.8, 120.1, 123.7, 131.3, 134.5, 135.9, 167.4, 169.3; HRMS calcd for $C_{19}H_{19}DN_2O_4$ 341.1486, found 341.1488.

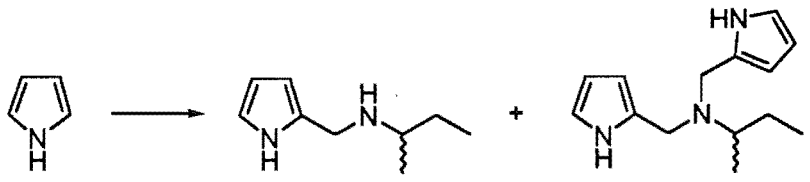
***R*-Alpine borane® sequence:** General procedure F was carried out using a sample of the deuterated *N*-leucinylpyrrole **4.13b** (22 mg, 0.07 mmol) and *R*-Alpine borane® (145 μL of 0.5 M solution in THF, 0.07 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.8b** and **4.8c** (20 mg, 90%, 1:>9 by $^1$H NMR) as a colourless oil which rapidly changed to a red colour when exposed to air. Spectral data as recorded above.

## 8.4.2. Procedure for the hydrolysis of unlabelled and [*methylene-d*$_1$]-labelled 2-hydroxymethyl-1-(*N*-phthalyl-*L*-leucinyl)pyrroles 4.8a-c




**General Procedure:** An equivalent of potassium hydroxide in $D_2O$ (approx 10 μL) was added to a solution of either **4.8a**, **4.8b** or **4.8c** (typically 3 mg) in $CD_3CN$ (150 μL) containing $CHCl_3$ as an internal standard (δ 7.25), and an equivalent of *S*-(+)-*sec*-butylamine (external nucleophile). The ratio of the *N*-acyl **4.8**, *O*-acyl **4.10** and aminomethylpyrroles **4.11** and **4.12** was then monitored by $^1$H NMR spectroscopy over time (*ca.* 18 h). See Table 4.1 in Chapter 4 for the NMR analysis.

### *8.4.4. Independent synthesis of aminomethylpyrroles 4.11 and 4.12*




***N*-(Pyrrol-2-ylmethyl)-(±)-*sec*-butylamine 4.11**

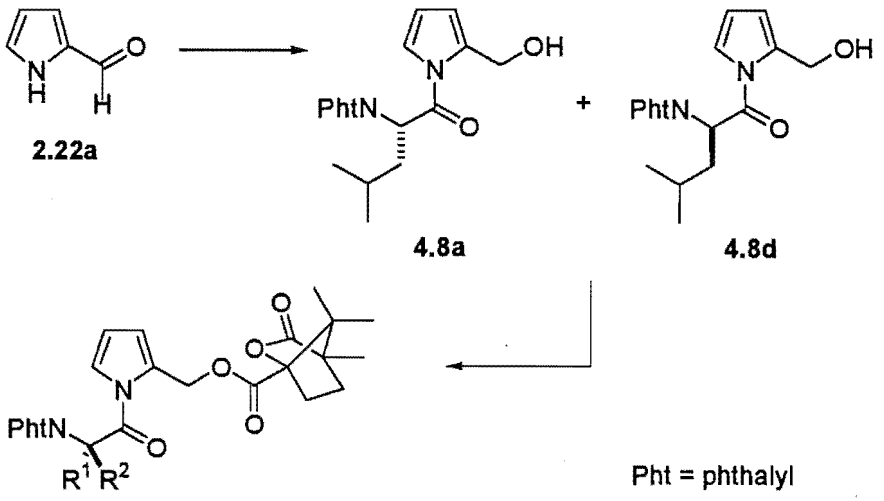
A solution of (±)-*sec*-butylamine hydrochloride (858 mg, 7.83 mmol, 1.05 equiv) in 37% aqueous formalin (586 μL, 7.83 mmol, 1.05 equiv) was added slowly to pyrrole (517 μL, 7.45 mmol). The mixture was stirred for 90 min and allowed to stand

overnight. It was then poured into 25% aqueous sodium hydroxide (20 mL) and extracted with ether (3x15 mL). The combined ethereal extracts were washed with water (2x15 mL), dried and evaporated under reduced pressure. The resulting residue was distilled under high vacuum to give **4.11** (704 mg, 62%) as a pale yellow oil: IR ($CHCl_3$) 3468, 3323, 1603, 1574 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, $J$ = 7.3 Hz, 3H, $CH_2$*Me*), 1.06 (d, $J$ = 6.3 Hz, 3H, CH*Me*), 1.34 (m, 1H, C$H_2$Me), 1.52 (m, 1H, C$H_2$Me), 2.62 (m, 1H, C*H*Me), 3.73 (ABq, $J$ = 13.2 Hz, 1H, C$H_2$N), 3.81 (ABq, $J$ = 13.2 Hz, 1H, C$H_2$N), 6.01 (m, 1H, pyrrole H4), 6.11 (m, 1H, pyrrole H3), 6.68 (m, 1H, pyrrole H5), 9.28 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 10.1, 19.6, 29.3, 44.1, 54.3, 105.9, 107.7, 117.1, 130.7; HRMS calcd for $C_9H_{16}N_2$ 152.1314, found 152.1313.

***N,N*-Di(pyrrol-2-ylmethyl)-(±)-*sec*-butylamine 4.12**

A solution of (±)-*sec*-butylamine hydrochloride (250 mg, 2.28 mmol) in 37% aqueous formalin (342 μL, 4.56 mmol, 2 equiv) was treated with pyrrole (317 μL, 4.56 mmol, 2 equiv) by the procedure described above for the preparation of **4.11**. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2 + 5% triethylamine) gave two compounds. The first to elute was **4.12** (104 mg, 20%) as a pale orange oil: IR ($CHCl_3$) 3472, 3389, 1570 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, $J$ = 7.3 Hz, 3H, $CH_2$*Me*), 0.99 (d, $J$ = 6.8 Hz, 3H, CH*Me*), 1.32 (m, 1H, C$H_2$Me), 1.57 (m, 1H, C$H_2$Me), 2.66 (m, 1H, C*H*Me), 3.49 (ABq, $J$ = 14.2 Hz, 2H, C$H_2$N), 3.65 (ABq, $J$ = 14.2 Hz, 2H, C$H_2$N), 6.02 (m, 2H, pyrrole H4), 6.10 (m, 2H, pyrrole H3), 6.69 (m, 2H, pyrrole H5), 8.25 (bs, 2H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 11.8, 13.5, 26.5, 46.6, 55.8, 106.9, 108.0, 117.0, 130.4; HRMS calcd for $C_{14}H_{21}N_3$ 231.1736, found 231.1735. The second compound to elute was **4.11** (265 mg, 76%) as a pale orange oil. Spectral data as recorded above.

## 8.4.3. Preparation of unlabelled and [*methylene-d*$_1$]-labelled camphanates 4.14a-c and 4.15a-c




**4.14a** $R^1 = CH_2CHMe_2$, $R^2 = H$
**4.15a** $R^1 = H$, $R^2 = CH_2CHMe_2$

**2-Hydroxymethyl-1-(*N*-phthalyl-*L*-leucinyl)pyrrole 4.8a and 2-hydroxymethyl-1-(*N*-phthalyl-*D*-leucinyl)pyrrole 4.8d**

Pyrrole-2-carboxaldehyde **2.22a** (20 mg, 0.21 mmol) was *N*-acylated with racemic *N*-phthalyl-*DL*-leucine acid fluoride (61 mg, 0.23 mmol, 1.1 equiv), by the method described for the preparation of **4.8a**. The resulting sample was then reduced with zinc borohydride (1.50 mL of 0.14 M solution in ether, 0.21 mmol, 1 equiv) by the method described in general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.8a** and **4.8d** (58 mg, 81% for 2 steps, 1:1 from analysis of camphanates prepared by method G below) as a colourless oil which rapidly changed to a red colour when exposed to air. Spectral data as recorded above.

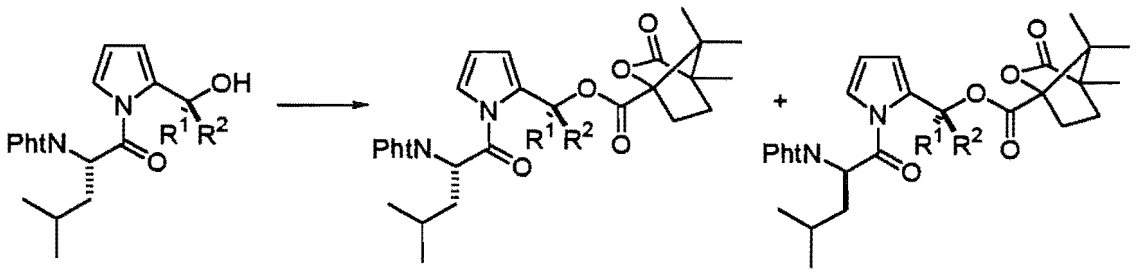
**(1*S*,4*R*)-1-(*N*-Phthalyl-*L*-leucinyl)pyrrol-2-ylmethyl camphanate 4.14a and (1*S*,4*R*)-1-(*N*-phthalyl-*D*-leucinyl)pyrrol-2-ylmethyl camphanate 4.15a**

General procedure G was carried out on a racemic sample of the *N*-leucinyl hydroxymethylpyrroles **4.8a** and **4.8d** (15 mg, 0.05 mmol, 1:1). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.14a** and **4.15a** (20 mg, 83%, 1:1) as a colourless oil: IR ($CHCl_3$) 1782, 1719, 1383 $cm^{-1}$; $^1H$ NMR ($CD_3CN$, 300

MHz) δ 0.63 (s, 3H, camph-Me), 0.73 (d, $J$ = 6.3 Hz, 3H, CH$Me_2$), 0.78 (s, 3H, camph-Me), 0.80 (d, $J$ = 6.3 Hz, 3H, CH$Me_2$), 0.82 (s, 3H, camph-Me), 1.35 – 1.44 (m, 2H, C$H$Me$_2$ & camph-CH$_2$), 1.67 – 1.80 (m, 3H, C$H_2$ & camph-CH$_2$), 2.08 – 2.25 (m, 2H, C$H_2$ & camph-CH$_2$), 5.10 (ABq, $J$ = 12.7 Hz, 0.5H, C$H_2$O- **4.15a**), 5.13 (ABq, $J$ = 12.7 Hz, 0.5H, C$H_2$O- **4.14a**), 5.19 (ABq, $J$ = 12.7 Hz, 0.5H, C$H_2$O- **4.14a**), 5.20 (ABq, $J$ = 12.7 Hz, 0.5H, C$H_2$O- **4.15a**), 5.36 (m, 1H, α$H$), 6.01 (m, 1H, pyrrole H4), 6.20 (m, 1H, pyrrole H3), 6.97 (m, 1H, pyrrole H5), 7.63 (m, 4H, Pht Hs); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.7, 16.6, 16.7, 21.3, 23.0, 25.1, 29.0, 30.6, 37.4, 51.5, 54.2, 54.8, 60.5, 91.2, 112.6, 116.7, 120.9, 123.7, 129.7, 131.4, 134.4, 167.1, 167.5, 167.6, 178.3; HRMS calcd for $C_{29}H_{32}N_2O_7$ 520.2210, found 520.2206.

The reaction was repeated as described in general procedure G using a sample of the *N*-leucinylhydroxymethylpyrrole **4.8a** (20 mg, 0.06 mmol). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.14a** and **4.15a** (29 mg, 94%, ~9:1) as a colourless oil. Selected $^1$H NMR ($CD_3CN$, 300 MHz) δ 5.10 (ABq, $J$ = 12.7 Hz, 0.1H, C$H_2$O- **4.15a**), 5.13 (ABq, $J$ = 12.7 Hz, 0.9H, C$H_2$O- **4.14a**), 5.19 (ABq, $J$ = 12.7 Hz, 0.9H, C$H_2$O- **4.14a**), 5.20 (ABq, $J$ = 12.7 Hz, 0.1H, C$H_2$O- **4.15a**).

The reaction was repeated using a sample of the *N*-leucinylhydroxymethylpyrrole **4.8a** (12 mg, 0.04 mmol) and a modified general procedure G in which the reaction mixture was stirred at rt for 48 h. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.14a** and **4.15a** (15 mg, 79%, ~5:1) as a colourless oil. Selected $^1$H NMR ($CD_3CN$, 300 MHz) δ 5.10 (ABq, $J$ = 12.7 Hz, 0.17H, C$H_2$O- **4.15a**), 5.13 (ABq, $J$ = 12.7 Hz, 0.83H, C$H_2$O- **4.14a**), 5.19 (ABq, $J$ = 12.7 Hz, 0.83H, C$H_2$O- **4.14a**), 5.20 (ABq, $J$ = 12.7 Hz, 0.17H, C$H_2$O- **4.15a**).



**4.8b** $R^1$ = D, $R^2$ = H
**4.8c** $R^1$ = H, $R^2$ = D

**4.14b** $R^1$ = D, $R^2$ = H
**4.14c** $R^1$ = H, $R^2$ = D

**4.15b** $R^1$ = D, $R^2$ = H
**4.15c** $R^1$ = H, $R^2$ = D

**[*methlyene-d*$_1$]-(1*S*,4*R*)-1-(*N*-Phthalyl-*L*-leucinyl)pyrrol-2-ylmethyl camphanate 4.14b and 4.14c and [*methlyene-d*$_1$]-(1*S*,4*R*)-1-(*N*-phthalyl-*D*-leucinyl)pyrrol-2-ylmethyl camphanate 4.15b and 4.15c**

A sample of **4.8b** and **4.8c** (9 mg, 0.03 mmol, >9:1) was reacted with (1*S*,4*R*)-(–)-camphanoyl chloride (7 mg, 0.03 mmol, 1.2 equiv) according to general method G. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.14b** and **4.15b**† (10 mg, 74%, 9:1) as a colourless oil: $^1$H NMR ($CD_3CN$, 300 MHz) δ 0.63 (s, 3H, camph-Me), 0.73 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 0.78 (s, 3H, camph-Me), 0.80 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 0.82 (s, 3H, camph-Me), 1.35 – 1.44 (m, 2H, C*H*Me$_2$ & camph-CH$_2$), 1.67 – 1.80 (m, 3H, C*H*$_2$ & camph-CH$_2$), 2.08 – 2.25 (m, 2H, C*H*$_2$ & camph-CH$_2$), 5.10 (s, 0.1H, C*H*DO- **4.15b**), 5.17 (s, 0.9H, C*H*DO- **4.14b**), 5.36 (m, 1H, α*H*), 6.01 (m, 1H, pyrrole H4), 6.20 (m, 1H, pyrrole H3), 6.97 (m, 1H, pyrrole H5), 7.63 (m, 4H, Pht Hs); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 9.7, 16.6, 16.7, 21.3, 23.0, 25.1, 29.0, 30.6, 37.4, 51.5, 54.2, 54.8, 60.2 (t, $J$ = 21.9 Hz), 91.2, 112.6, 116.7, 120.9, 123.7, 129.7, 131.4, 134.4, 167.1, 167.5, 167.6, 178.3; HRMS calcd for $C_{29}H_{31}DN_2O_7$ 521.2271, found 521.2278.

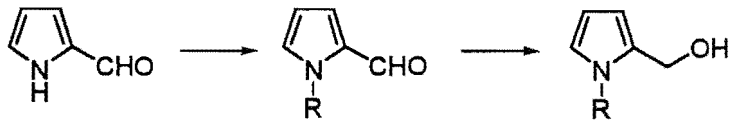
The reaction was repeated using a sample of **4.8b** and **4.8c** (12 mg, 0.03 mmol, 1:>9). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave a mixture of **4.14c** and **4.15c**† (15 mg, 86%, 9:1) as a colourless oil. Selected $^1$H NMR ($CD_3CN$, 300 MHz) δ 5.13 (s, 0.9H, C*H*DO- **4.14c**), 5.19 (s, 0.1H, C*H*DO- **4.15c**).

† Also present in trace amounts (<5% by $^1$H NMR) were the corresponding deuterated isomers. For ease of analysis these isomers have been omitted.

# 8.5 Experimental Work Described in Chapter Five

## 8.5.1. Determination of optimal pyrrole *N*-substituent and leaving group

### 8.5.1.1. Preparation of 2-hydroxymethyl-1-(substituted)pyrroles **5.3a-d**




**2.22a** **5.2a-d** **5.3a-d**

| | R |
|---|---|
| **a** | $CO(CH_2)_2Ph$ |
| **b** | $SO_2Ph$ |
| **c** | $SO_2CH{:}CHPh$ |
| **d** | $SO_2(CH_2)_2Ph$ |

**1-(3-Phenylpropionyl)pyrrole-2-carboxaldehyde 5.2a**

General procedure C was carried out using pyrrole-2-carboxaldehyde **2.22a** (60 mg, 0.63 mmol) and hydrocinnamoyl chloride[20] (103 μL, 0.69 mmol, 1.1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave **5.2a** (113 mg, 79%) as a white solid: mp 88 °C (lit.[21] 89 – 90 °C); IR ($CHCl_3$) 1730, 1665 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.14 (m, 2H, $CH_2Ph$), 3.26 (m, 2H, $COCH_2$), 6.34 (m, 1H, pyrrole H4), 7.20 – 7.35 (m, 7H, pyrrole H3 & pyrrole H5 & phenyl Hs), 10.30 (s, 1H, CHO); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 29.9, 37.2, 112.5, 122.1, 125.8, 126.2, 128.1, 128.3, 135.2, 139.4, 171.0, 182.1; HRMS calcd for $C_{14}H_{13}NO_2$ 227.0946, found 227.0946.

**1-(Phenylsulfonyl)pyrrole-2-carboxaldehyde 5.2b**

General procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (50 mg, 0.53 mmol) and phenylsulfonyl chloride (81 μL, 0.63 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **5.2b** (121 mg, 97%) as a white solid: mp 81 °C (lit.[22] 79.5 – 80.5 °C); IR ($CHCl_3$) 1688, 1672, 1379, 1178 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 6.43 (m, 1H, pyrrole H4), 7.17 (m, 1H, pyrrole H3), 7.52 – 7.68 (m, 4H, pyrrole H5 & phenyl Hs), 7.93 (m, 2H, phenyl Hs), 9.95 (s, 1H, CHO); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 112.5, 124.8, 127.4, 129.5, 133.5, 134.5, 138.1, 178.7; HRMS calcd for $C_{11}H_9NO_3S$ 235.0303, found 235.0303.

**1-(β-*trans*-Styrenesulfonyl)pyrrole-2-carboxaldehyde 5.2c**

The modified general procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (50 mg, 0.53 mmol) and β-*trans*-styrenesulfonyl chloride (128 mg, 0.63 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **5.2c** (77 mg, 56%) as a pale yellow solid. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give a white crystalline solid: mp 88 °C; IR ($CHCl_3$) 2837, 1682, 1612, 1377, 1167 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 6.41 (m, 1H, pyrrole H4), 7.21 (m, 1H, pyrrole H3), 7.40 – 7.47 (m, 4H, C*H*Ph & phenyl Hs), 7.54 (m, 2H, phenyl Hs), 7.62 (m, 1H, pyrrole H5), 7.81 (d, $J$ = 15.6 Hz, 1H, $SO_2CH$), 9.79 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 111.6, 123.5, 127.5, 128.9, 129.1, 129.6, 131.4, 132.0, 133.1, 146.0, 178.2; HRMS calcd for $C_{13}H_{11}NO$ (M-$SO_2$) 197.0841, found 197.0841. Anal. Calcd for $C_{13}H_{11}NO_3S$: C, 59.76; H, 4.24; N, 5.36. Found: C, 59.74; H, 4.16; N, 5.30.

**1-(2-Phenylethanesulfonyl)pyrrole-2-carboxaldehyde 5.2d**

To a suspension of sodium 2-phenylethylsulfonate[23] (5.00 g, 24.0 mmol) in phosphorus oxychloride (8 mL) was added powdered phosphorus pentachloride (5.00g, 24.0 mmol, 1 equiv) portionwise. The mixture was then stirred vigorously at 70 °C for 2½ h under $N_2$. The mixture was allowed to cool to rt and was extracted with dichloromethane (4x20 mL). The organic phase was dried and evaporated under reduced pressure to give the crude product which was obtained by recrystallisation from petroleum ether to give 2-phenylethanesulfonyl chloride (3.72 g, 76%) as a white solid: mp 33 °C (lit.[23] 34 °C); IR ($CHCl_3$) 1377, 1167 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.33 (m, 2H, C$H_2$Ph), 3.90 (m, 2H, C$H_2SO_2Cl$), 7.22 – 7.38 (m, 5H, phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 30.4, 66.1, 127.6, 128.5, 129.1, 135.5; LRMS *m/z* (rel. intensity) 206 (18), 204 (48), 105 (100), 104 (97).

The modified general procedure B was carried out using pyrrole-2-carboxaldehyde **2.22a** (50 mg, 0.53 mmol) and 2-phenylethanesulfonyl chloride (129 mg, 0.63 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **5.2d** (116 mg, 84%) as a pale pink solid: mp 61 – 63 °C; IR ($CHCl_3$) 1680, 1377, 1169 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.08 (m, 2H, C$H_2$Ph), 4.10 (m, 2H, $SO_2CH_2$), 6.38 (m, 1H, pyrrole H4), 7.12 – 7.30 (m, 6H, pyrrole H3 & phenyl Hs), 7.56

(m, 1H, pyrrole H5), 9.68 (s, 1H, C*H*O); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 29.3, 55.8, 111.5, 127.1, 128.2, 128.8, 129.0, 131.0, 133.1, 136.2, 178.2; HRMS calcd for $C_{13}H_{13}NO$ (M-$SO_2$) 199.0997, found 199.0994.

**2-Hydroxymethyl-1-(3-phenylpropionyl)pyrrole 5.3a**

The *N*-hydrocinnamoylpyrrole **5.2a** (50 mg, 0.22 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **5.3a** (47 mg, 93%) as a pale orange oil: IR ($CHCl_3$) 3533, 1707, 1412, 1317 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.09 (m, 2H, C$H_2$Ph), 3.19 (m, 2H, COC$H_2$), 3.77 (bs, 1H, O*H*), 4.63 (s, 2H, C$H_2$OH), 6.18 (m, 1H, pyrrole H4), 6.22 (m, 1H, pyrrole H3), 7.08 (m, 1H, pyrrole H5), 7.21 – 7.35 (m, 5H, phenyl Hs); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 30.4, 37.4, 57.9, 112.2, 114.6, 120.8, 126.5, 128.3, 128.7, 135.5, 139.9, 172.2; HRMS calcd for $C_{14}H_{15}NO_2$ 229.1103, found 229.1103.

**2-Hydroxymethyl-1-(phenylsulfonyl)pyrrole 5.3b**

The *N*-phenylsulfonylpyrrole **5.2b** (50 mg, 0.21 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.3b** (46 mg, 92%) as a pale pink solid: mp 41 – 42 °C; IR ($CHCl_3$) 3578, 1369, 1177 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 2.57 (bs, 1H, O*H*), 4.61 (s, 2H, C$H_2$OH), 6.24 – 6.28 (m, 2H, pyrrole H4 & pyrrole H3), 7.28 (m, 1H, pyrrole H5), 7.49 – 7.65 (m, 3H, phenyl Hs), 7.82 (m, 2H, phenyl Hs); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 56.8, 112.0, 115.3, 123.6, 126.6, 129.5, 134.0, 134.6, 139.0; HRMS calcd for $C_{11}H_{11}NO_3S$ 237.0460, found 237.0458.

**2-Hydroxymethyl-1-(β-*trans*-styrenesulfonyl)pyrrole 5.3c**

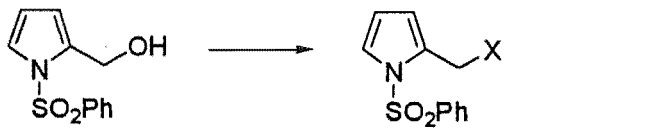
The *N*-styrenesulfonylpyrrole **5.2c** (50 mg, 0.19 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **5.3c** (48 mg, 95%) as a pink solid: mp 76 – 77 °C; IR ($CHCl_3$) 3585, 3026, 1614, 1369, 1173 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 2.64 (bs, 1H, O*H*), 4.73 (s, 2H, C$H_2$OH), 6.24 (m, 1H, pyrrole H4), 6.30 (m, 1H, pyrrole H3), 7.02 (d, $J$ = 15.6 Hz, 1H, C*H*Ph), 7.18 (m, 1H, pyrrole H5), 7.36 – 7.48 (m, 5H, phenyl Hs), 7.63 (d, $J$ = 15.6 Hz, 1H, $SO_2$C*H*); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 56.7, 111.6, 115.2, 123.0,

124.3, 128.7, 129.1, 131.5, 131.7, 134.1, 143.4; HRMS calcd for $C_{13}H_{13}NO_3S$ 263.0616, found 263.0616.

**2-Hydroxymethyl-1-(2-phenylethanesulfonyl)pyrrole 5.3d**

The *N*-phenylethanesulfonylpyrrole **5.2d** (50 mg, 0.19 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **5.3d** (47 mg, 94%) as a colourless oil: IR ($CHCl_3$) 3578, 1369, 1175 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.47 (bs, 1H, O*H*), 2.99 (m, 2H, C*H*$_2$Ph), 3.70 (m, 2H, $SO_2CH_2$), 4.76 (s, 2H, C*H*$_2$OH), 6.23 (m, 1H, pyrrole H4), 6.32 (m, 1H, pyrrole H3), 7.11 – 7.31 (m, 6H, pyrrole H5 & phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 29.3, 56.7 (2xC), 111.1, 115.6, 124.0, 127.2, 128.3, 128.9, 133.8, 136.6; HRMS calcd for $C_{13}H_{15}NO_3S$ 265.0773, found 265.0771.

## 8.5.1.2. Preparation of 2-substituted pyrroles **5.6a** and **5.6b**




**5.3b**

**5.6a** X = Cl
**5.6b** X = *N*-Pht-*L*-LeuCO$_2$

**2-Chloromethyl-1-(phenylsulfonyl)pyrrole 5.6a**

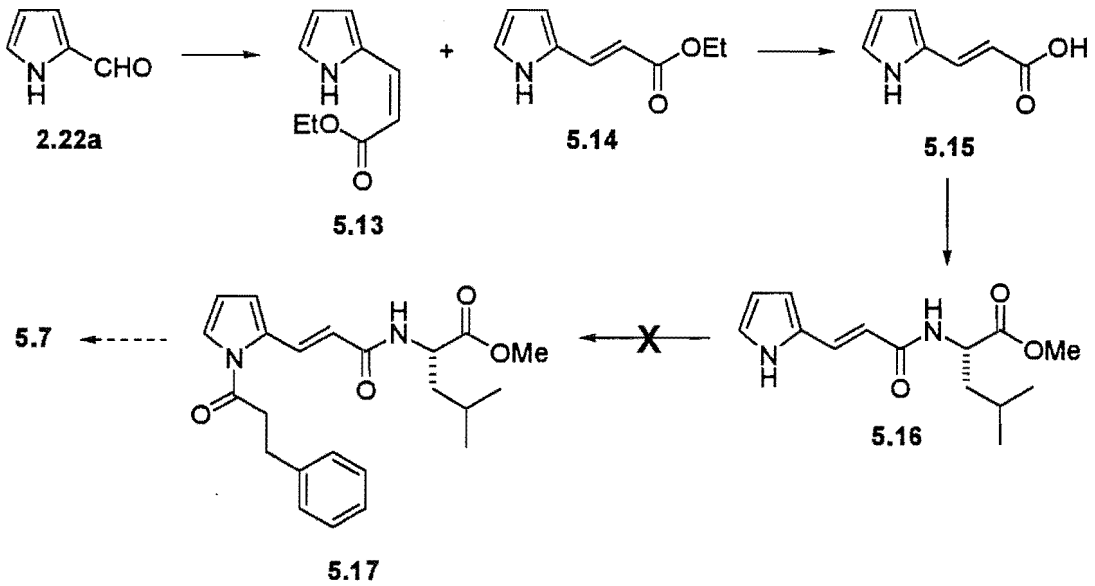
General procedure I was carried out using the *N*-phenylsulfonylpyrrole **5.3b** (30 mg, 0.13 mmol) and methanesulfonyl chloride (15 μL, 0.19 mmol, 1.5 equiv). The residual oil was eluted through a short plug of silica (ethyl acetate/petroleum ether, 1:1) to give **5.6a** (quantitative) as a white solid: mp 66 °C; IR ($CHCl_3$) 1371, 1178 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 4.84 (s, 2H, C*H*$_2$Cl), 6.26 (m, 1H, pyrrole H4), 6.38 (m, 1H, pyrrole H3), 7.34 (m, 1H, pyrrole H5), 7.48 – 7.64 (m, 3H, phenyl Hs), 7.89 (m, 2H, phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 37.2, 111.8, 117.1, 124.5, 127.1, 129.3, 134.0, 138.8, 142.6; HRMS calcd for $C_{11}H_{10}NO_2S$ (M-Cl) 220.0432, found 220.0435.

**1-(Phenylsulfonyl)pyrrol-2-ylmethyl *N*-phthalyl-*L*-leucine 5.6b**

The hydroxymethylpyrrole **5.3b** (30 mg, 0.13 mmol), DMAP (15 mg, 0.13 mmol, 1 equiv) and Hünig's base (26 μL, 0.15 mmol, 1.2 equiv) were dissolved in dichloromethane (8 mL) at rt. *N*-Phthalyl-*L*-leucine acid fluoride (40 mg, 0.15 mmol, 1.2 equiv) dissolved in dichloromethane (2 mL) was added and the resultant solution was stirred for 24 h under $N_2$. Ethyl acetate (10 mL) was added and the mixture was washed with 10% aqueous citric acid (10 mL), water (2x10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.6b** (55 mg, 90%) as a colourless oil which solidified at 0 °C. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give a white solid: mp 112 °C; IR ($CHCl_3$) 1776, 1742, 1717, 1389, 1371, 1186 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 0.89 (d, $J$ = 6.3 Hz, 3H, CH*Me*$_2$), 1.43 (m, 1H, C*H*Me$_2$) 1.75 (m, 1H, C*H*$_2$CHMe$_2$), 2.29 (m, 1H, C*H*$_2$CHMe$_2$), 4.80 (m, 1H, α*H*), 5.30 (m, 2H, C*H*$_2$O-Leu), 6.25 (m, 1H, pyrrole H4), 6.37 (m, 1H, pyrrole H3), 7.32 (m, 1H, pyrrole H5), 7.52 – 7.87 (m, 9H, Pht Hs & phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 20.9, 23.1, 25.0, 37.0, 50.7, 58.8, 111.8, 117.9, 123.5, 124.3, 126.7, 128.3, 129.5, 131.8, 134.0, 134.1, 139.2, 167.7, 169.4; HRMS calcd for $C_{25}H_{24}N_2O_6S$ 480.1355, found 480.1361; Anal. Calcd for $C_{25}H_{24}N_2O_6S$: C, 62.49; H, 5.03; N, 5.83. Found: C, 62.50; H, 5.33; N, 5.96.

## 8.5.2. Preparation of pyrrole-based peptidomimetics

### 8.5.2.1. Attempted preparation of peptidomimetics of type **5.7**




**Ethyl (*E*)-3-(1*H*-pyrrol-2-yl)-2-propenoate 5.14**

Pyrrole-2-carboxaldehyde **2.22a** (2.02g, 21.2 mmol) and (ethoxycarbonyl)methylene triphenylphosphorane[24] (7.40 g, 21.2 mmol, 1 equiv) in benzene (80 mL) were heated at reflux for 2 h and then allowed to stand at rt for 12 h. The benzene was removed under reduced pressure and the residual oil was purified by distillation under reduced pressure followed by flash chromatography on silica (ethyl acetate/petroleum ether, 1:10 to 1:1) to give two compounds. The first to elute was the *cis*-isomer **5.13** (0.60 g, 17%) as a yellow oil: IR ($CHCl_3$) 3279, 1686, 1601 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, $J$ = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 4.18 (q, $J$ = 7.1 Hz, 2H, $CO_2CH_2$), 5.50 (d, $J$ = 12.6 Hz, 1H, $CHCO_2$), 6.24 (m, 1H, pyrrole H4), 6.48 (m, 1H, pyrrole H3), 6.72 (d, $J$ = 12.6 Hz, 1H, $CHCHCO_2$), 6.96 (m, 1H, pyrrole H5), 12.25 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 60.2, 107.5, 110.0, 118.5, 122.7, 129.0, 134.5, 169.0. The second compound to elute was the *trans*-isomer **5.14** (1.70 g, 49%) as a pale yellow solid: mp 51 – 52 °C (lit.[25] 54 – 56 °C); IR ($CHCl_3$) 3475, 3321, 1692, 1628 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 4.24 (q,

$J$ = 7.3 Hz, 2H, $CO_2CH_2$), 6.08 (d, $J$ = 15.6 Hz, 1H, $CHCO_2$), 6.27 (m, 1H, pyrrole H4), 6.56 (m, 1H, pyrrole H3), 6.92 (m, 1H, pyrrole H5), 7.58 (d, $J$ = 15.6 Hz, 1H, $CHCHCO_2$), 9.29 (bs, 1H, N*H*); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 14.3, 60.3, 110.8, 111.0, 114.2, 122.5, 128.4, 134.5, 168.1; HRMS calcd for $C_9H_{11}NO_2$ 165.0790, found 165.0790.

**(*E*)-3-(1*H*-Pyrrol-2-yl)-2-propenoic acid 5.15**

The pyrrole ester **5.14** (201 mg, 1.22 mmol) was hydrolysed with sodium hydroxide by general procedure J. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.15** (147 mg, 88%) as a pale yellow solid: mp >155 °C decomp.; (lit.[26] 150 – 200 °C decomp.); IR ($CHCl_3$) 3475, 1695, 1630 cm$^{-1}$; $^1$H NMR ($CD_3OD$, 300 MHz) δ 6.04 (d, $J$ = 15.6 Hz, 1H, $CHCO_2$), 6.17 (m, 1H, pyrrole H4), 6.48 (m, 1H, pyrrole H3), 6.90 (m, 1H, pyrrole H5), 7.51 (d, $J$ = 15.6 Hz, 1H, $CHCHCO_2$), 10.99 (bs, 1H, N*H*); $^{13}$C NMR ($CD_3OD$, 75 MHz) δ 111.2, 111.5, 116.2, 124.6, 130.0, 137.1, 172.1; HRMS calcd for $C_7H_7NO_2$ 137.0477, found 137.0474.

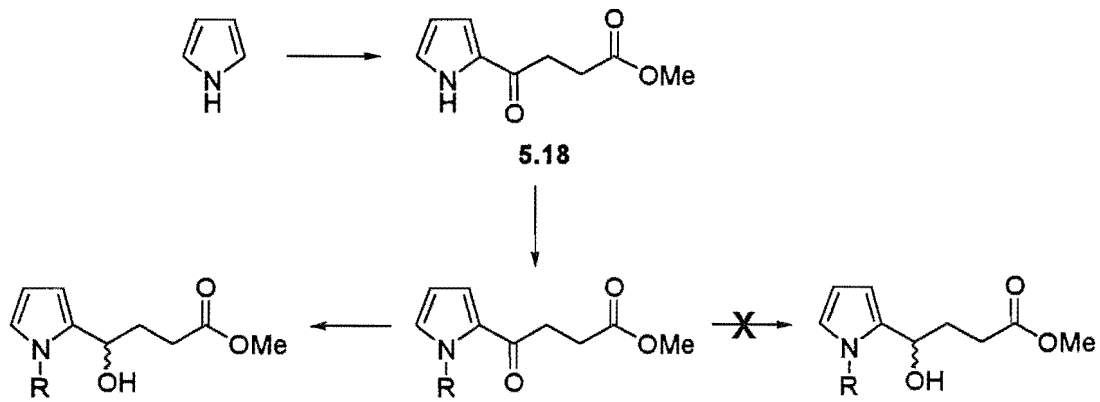
***N*-[(*E*)-3-(1*H*-Pyrrol-2-yl)-2-propenoyl]-*L*-leucine methyl ester 5.16**

The pyrrole acid **5.15** (127 mg, 0.92 mmol) was coupled with *L*-leucine methyl ester hydrochloride (168 mg, 0.92 mmol, 1 equiv) according to general procedure K. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2 then 1:1) gave **5.16** (224 mg, 92%) as a yellow oil: IR ($CHCl_3$) 3476, 3439, 3315, 1736, 1663, 1618, 1510 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.92 (d, $J$ = 5.9 Hz, 3H, $CHMe_2$), 0.93 (d, $J$ = 6.3 Hz, 3H, $CHMe_2$), 1.60 – 1.77 (m, 3H, $CH_2CHMe_2$), 3.70 (s, 3H, $CO_2Me$), 4.65 (m, 1H, α*H*), 6.15 – 6.20 (m, 2H, pyrrole H4 & $CHCO_2$), 6.37 (m, 1H, pyrrole H3 ), 6.85 (m, 1H, pyrrole H5), 7.17 (d, $J$ = 7.3 Hz, 1H, CON*H*), 7.45 (d, $J$ = 15.6 Hz, 1H, $CHCHCO_2$), 10.12 (bs, 1H, N*H*); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 21.7, 22.7, 24.8, 41.0, 51.2, 52.3, 110.2, 112.6, 113.4, 122.1, 128.5, 132.2, 167.7, 174.3; HRMS calcd for $C_{14}H_{20}N_2O_3$ 264.1474, found 264.1473.

**Attempted preparation of the *N*-hydrocinnamoylpyrrole 5.17**

Attempts to N-substitute **5.16** (typically 81 mg, 0.31 mmol) with hydrocinnamoyl chloride (1.4 equiv) by general procedure B proved unsuccessful, giving returned starting material **5.16**. Similarly, an attempt to N-substitute **5.16** (63 mg, 0.24 mmol) with hydrocinnamoyl chloride (1.1 equiv) by general procedure C also proved unsuccessful, with the $^1$H NMR spectrum of the crude reaction work up showing returned starting material **5.16**, with no evidence of any of the desired product **5.17**.

## 8.5.2.2. Preparation of peptidomimetics of type **5.8** and **5.9**




**Methyl 4-oxo-4-(1*H*-pyrrol-2-yl)butanoate 5.18**

A solution of pyrrole (1.50 g, 22.4 mmol) in ether (9 mL) was added dropwise to a solution of methylmagnesium iodide (11.74 mL of 2.0 M solution in ether, 23.5 mmol, 1.05 equiv) under $N_2$ so as to cause slight reflux. The solution was then heated at reflux for an additional 30 min. After cooling to rt, methyl succinyl chloride[27] (2.75 mL, 22.4 mmol, 1 equiv) in ether (6 mL) was added, and the resulting mixture was heated at reflux for 1 h. The reaction was left overnight, and then poured into cold water. The aqueous and ether layers were separated, and the aqueous phase was extracted with ether (3x10 mL). The combined ethereal layers were washed with water (2x10 mL), dried and evaporated under reduced pressure. The resulting residue was distilled at reduced pressure (oil pump) to give **5.18** (1.97 g, 49%) as yellow solid. An analytical sample was obtained by recrystallisation from petroleum ether to give white crystals: mp 49 °C; IR

($CHCl_3$) 3452, 3285, 1736, 1647 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.74 (t, $J$ = 6.8 Hz, 2H, $CH_2CO_2Me$), 3.16 (t, $J$ = 6.8 Hz, 2H, $COCH_2$), 3.69 (s, 3H, $CO_2Me$), 6.27 (m, 1H, pyrrole H4), 6.99 (m, 1H, pyrrole H3 ), 7.05 (m, 1H, pyrrole H5), 10.20 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 28.2, 32.4, 51.7, 110.6, 116.6, 125.1, 131.3, 173.3, 188.5; HRMS calcd for $C_9H_{11}NO_3$ 181.0739, found 181.0740. Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.62; H, 6.07; N, 7.58.

**Methyl 4-oxo-4-[1-(methanesulfonyl)pyrrol-2-yl]butanoate 5.19a**

General procedure B was carried out using the pyrrole **5.18** (60 mg, 0.33 mmol) and methanesulfonyl chloride (37 μL, 0.46 mmol, 1.4 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2), followed by recrystallisation from petroleum ether, gave **5.19a** (74 mg, 86%) as fine white needles: mp 92 °C; IR ($CHCl_3$) 1734, 1676, 1367, 1175 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.75 (t, $J$ = 6.6 Hz, 2H, $CH_2CO_2Me$), 3.18 (t, $J$ = 6.6 Hz, 2H, $COCH_2$), 3.70 (s, 3H, $CO_2Me$), 3.72 (s, 3H, $SO_2Me$), 6.31 (m, 1H, pyrrole H4), 7.21 (m, 1H, pyrrole H3), 7.57 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 27.6, 33.6, 42.8, 51.6, 110.0, 124.1, 129.6, 132.3, 172.9, 187.6; HRMS calcd for $C_{10}H_{13}NO_5S$ 259.0515, found 259.0511.

**Methyl 4-oxo-4-[1-(phenylsulfonyl)pyrrol-2-yl]butanoate 5.19b**

General procedure B was carried out using the pyrrole **5.18** (60 mg, 0.33 mmol) and phenylsulfonyl chloride (51 μL, 0.40 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2), followed by recrystallisation from ethyl acetate/petroleum ether, gave **5.19b** (46 mg, 43%) as fine white crystals: mp 139 – 140 °C; IR ($CHCl_3$) 1734, 1682, 1371, 1175 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.61 (t, $J$ = 6.8 Hz, 2H, $CH_2CO_2Me$), 3.03 (t, $J$ = 6.8 Hz, 2H, $COCH_2$), 3.61 (s, 3H, $CO_2Me$), 6.35 (m, 1H, pyrrole H4), 7.12 (m, 1H, pyrrole H3), 7.48 – 7.62 (m, 3H, phenyl Hs), 7.80 (m, 1H, pyrrole H5), 7.97 (m, 2H, phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 27.9, 33.8, 51.7, 110.5, 123.6, 128.1, 128.7, 130.2, 132.8, 133.6, 138.8, 142.7; HRMS calcd for $C_{15}H_{15}NO_5S$ 321.0671, found 321.0679.

**Methyl 4-oxo-4-[1-(3-phenylpropionyl)pyrrol-2-yl]butanoate 5.19c**

General procedure B was carried out using the pyrrole **5.18** (60 mg, 0.33 mmol) and hydrocinnamoyl chloride (59 μL, 0.40 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) gave **5.19c** (86 mg, 82%) as a pale yellow oil: IR ($CHCl_3$) 1734, 1649 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.73 (t, $J$ = 6.6 Hz, 2H, $CH_2CO_2Me$), 3.06 – 3.13 (m, 6H, $COCH_2$ & $CH_2CH_2Ph$), 3.62 (s, 3H, $CO_2Me$), 6.20 (m, 1H, pyrrole H4), 7.03 (m, 1H, pyrrole H3), 7.17 – 7.29 (m, 6H, pyrrole H5 & phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 28.1, 31.1, 33.9, 39.6, 51.7, 110.6, 122.7, 126.2, 127.6, 128.3, 128.4, 132.9, 140.0, 173.0, 173.4, 189.2; LRMS (FAB) *m/z* (rel. intensity) 312 (16), 182 (100), 181 (86), 150 (96).

**Methyl 4-hydroxy-4-[1-(methanesulfonyl)pyrrol-2-yl]butanoate 5.8a**

The *N*-mesylpyrrole **5.19a** (51 mg, 0.20 mmol) was reduced with sodium borohydride by general procedure E. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.8a** (44 mg, 85%) as a colourless oil which solidified at 0 °C. An analytical sample was obtained by recrystallisation from petroleum ether to give white needles: mp 70 °C; IR ($CHCl_3$) 3580, 1732, 1366, 1178 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.24 (m, 2H, $CHOHCH_2$), 2.57 (m, 2H, $CH_2CO_2Me$), 2.98 (bs, 1H, *OH*), 3.29 (s, 3H, $SO_2Me$), 3.70 (s, 3H, $CO_2Me$), 5.06 (t, $J$ = 6.6 Hz, 1H, *CH*OH), 6.25 (m, 1H, pyrrole H4), 6.34 (m, 1H, pyrrole H3), 7.15 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 30.2, 30.7, 43.0, 51.8, 64.8, 111.2, 112.2, 123.1, 136.7, 174.2; HRMS calcd for $C_{10}H_{15}NO_5S$ 261.0671, found 261.0671.

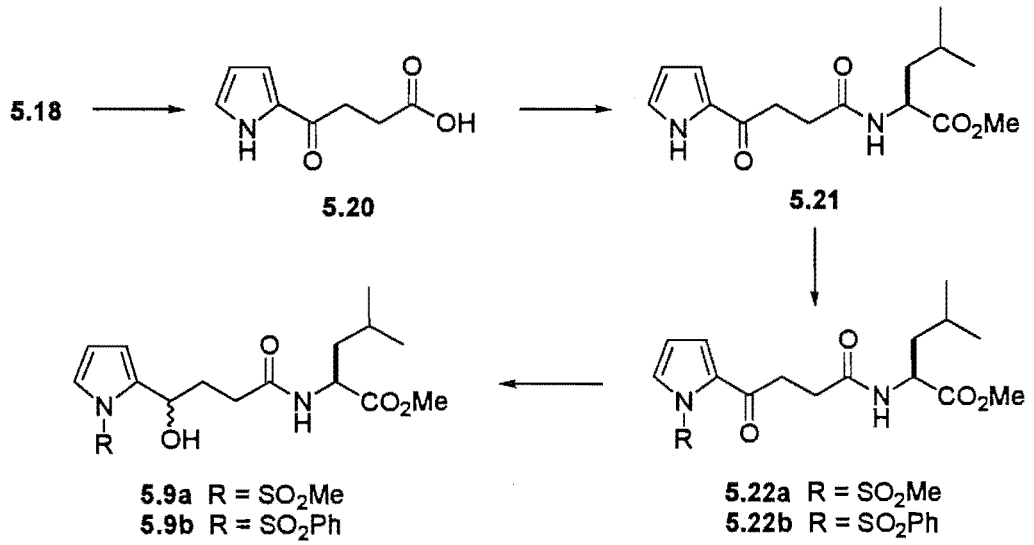
**Methyl 4-hydroxy-4-[1-(phenylsulfonyl)pyrrol-2-yl]butanoate 5.8b**

The *N*-phenylsulfonylpyrrole **5.19b** (25 mg, 0.08 mmol) was reduced with sodium borohydride by general procedure E. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) gave **5.8b** (23 mg, 90%) as a pale purple oil: IR ($CHCl_3$) 3578, 1776, 1732, 1367, 1177 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.06 (m, 2H, $CHOHCH_2$), 2.40 (m, 2H, $CH_2CO_2Me$), 3.59 (s, 3H, $CO_2Me$), 4.84 (m, 1H, *CH*OH), 6.19 (m, 1H, pyrrole H4), 6.25 (m, 1H, pyrrole H3), 7.22 (m, 1H, pyrrole H5), 7.42 – 7.57 (m, 3H, phenyl Hs), 7.71 (m, 2H, phenyl Hs); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 30.2, 30.6, 51.6,

64.7, 111.9, 112.6, 123.6, 126.5, 129.5, 134.0, 137.6, 139.1, 173.9; HRMS calcd for $C_{15}H_{15}NO_4S$ (M-$H_2O$) 305.0722, found 305.0723.

**Attempted reduction of the *N*-hydrocinnamoylpyrrole 5.19c**

The pyrrole **5.19c** (33 mg, 0.10 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave an inseparable mixture of predominantly deacylated starting material **5.18** with a small amount of the desired product **5.8c** (**5 : 2** by $^1$H NMR). Selected $^1$H NMR ($CDCl_3$, 300 MHz) of **5.8c** assigned from the mixture, δ 1.89 (m, 4H), 2.18 (m, 2H), 2.55 (m, 2H), 4.25 (m, 1H), 4.84 (m, 1H), 6.18 (m, 1H), 7.07 (m, 1H).




**4-Oxo-4-(1*H*-pyrrol-2-yl)butanoic acid 5.20**

The pyrrole ester **5.18** (300 mg, 1.66 mmol) was hydrolysed with sodium hydroxide by general procedure J to give **5.20** (217 mg, 78%) as a yellow solid: mp 141 °C (lit.[28] 140 – 141 °C); IR ($CHCl_3$) 3452, 1717, 1649, 1410 cm$^{-1}$; $^1$H NMR (acetone-$d_6$, 300 MHz) δ 2.79 (t, $J$ = 6.6 Hz, 2H, $CH_2CO_2H$), 3.22 (t, $J$ = 6.6 Hz, 2H, $COCH_2$), 6.35 (m, 1H, pyrrole H4), 7.14 (m, 1H, pyrrole H3 ), 7.23 (m, 1H, pyrrole H5), 10.99 (bs, 1H, N*H*); $^{13}$C NMR (acetone-$d_6$, 75 MHz) δ 27.7, 32.4, 110.0, 115.9, 124.8, 132.0, 173.6, 188.0; HRMS calcd for $C_8H_9NO_3$ 167.0582, found 167.0582.

**_N_-[4-Oxo-4-(1_H_-pyrrol-2-yl)]butanoyl-_L_-leucine methyl ester 5.21**

The pyrrole acid **5.20** (190 mg, 1.14 mmol) was coupled with _L_-leucine methyl ester hydrochloride (227 mg, 1.25 mmol, 1.1 equiv) according to general procedure K. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.21** (312 mg, 93%) as a mauve solid. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give colourless crystals: mp 100 – 101 °C; IR ($CHCl_3$) 3450, 3344, 1740, 1672, 1645, 1514 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.91 (d, $J$ = 5.9 Hz, 6H, CH$Me_2$), 1.51 – 1.67 (m, 3H, C$H_2$C$H$Me$_2$), 2.68 (m, 2H, C$H_2$CONH), 3.18 (m, 2H, COC$H_2$), 3.72 (s, 3H, $CO_2Me$), 4.66 (m, 1H, α$H$), 6.27 (m, 1H, pyrrole H4), 6.77 (d, $J$ = 7.8 Hz, 1H, CON$H$), 6.98 (m, 1H, pyrrole H3 ), 7.04 (m, 1H, pyrrole H5), 10.02 (bs, 1H, N$H$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.9, 22.7, 24.8, 30.3, 33.0, 41.7, 50.6, 52.2, 110.6, 116.8, 124.9, 131.4, 172.0, 173.9, 189.2; HRMS calcd for $C_{15}H_{22}N_2O_4$ 294.1580, found 294.1582. Anal. Calcd for $C_{15}H_{22}N_2O_4$: C, 61.21; H, 7.53; N, 9.52. Found: C, 61.09; H, 7.60; N, 9.46.

**_N_-{4-Oxo-4-[1-(methanesulfonyl)pyrrol-2-yl]}butanoyl-_L_-leucine methyl ester 5.22a**

General procedure B was carried out using the pyrrole **5.21** (60 mg, 0.20 mmol) and methanesulfonyl chloride (23 μL, 0.29 mmol, 1.4 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 2:1) gave **5.22a** (38 mg, 50%) as a white solid. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give a white solid: mp 106 – 107 °C; IR ($CHCl_3$) 3435, 1740, 1672, 1514, 1367, 1175 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.92 (d, $J$ = 5.9 Hz, 3H, CH$Me_2$), 0.93 (d, $J$ = 6.3 Hz, 3H, CH$Me_2$), 1.51 – 1.71 (m, 3H, C$H_2$C$H$Me$_2$), 2.65 (m, 2H, C$H_2$CONH), 3.22 (m, 2H, COC$H_2$), 3.71 (s, 3H, $CO_2Me$), 3.72 (s, 3H, $SO_2Me$), 4.61 (m, 1H, α$H$), 6.11 (d, $J$ = 8.3 Hz, 1H, CON$H$), 6.30 (m, 1H, pyrrole H4), 7.22 (m, 1H, pyrrole H3 ), 7.57 (m, 1H, pyrrole H5); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.9, 22.7, 24.8, 29.9, 34.3, 41.6, 43.1, 50.8, 52.2, 110.3, 124.5, 129.9, 132.5, 171.5, 173.4, 188.4; HRMS calcd for $C_{16}H_{24}N_2O_6S$ 372.1355, found 372.1347.

**_N_-{4-Hydroxy-4-[1-(methanesulfonyl)pyrrol-2-yl]}butanoyl-_L_-leucine methyl ester 5.9a**

The _N_-mesylpyrrole **5.22a** (54 mg, 0.14 mmol) was reduced with sodium borohydride by general procedure E. Flash chromatography on silica (ethyl acetate/petroleum ether, 5:2) gave **5.9a** (48 mg, 89%), which was a mixture of diastereomers (**2** : **1** by $^1$H NMR), as a pink oil: IR ($CHCl_3$) 3570, 3433, 3306, 1740, 1666, 1514, 1366, 1178 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) of major isomer, δ 0.94 (d, $J$ = 6.3 Hz, 6H, CH$Me_2$), 1.51 – 1.68 (m, 3H, C$H_2$C$H$Me$_2$), 2.24 (m, 2H, CHOHC$H_2$), 2.51 (m, 2H, C$H_2$CONH), 3.32 (s, 3H, $SO_2Me$), 3.74 (s, 3H, $CO_2Me$), 4.62 (m, 1H, α$H$), 5.07 (m, 1H, C$H$OH), 6.21 – 6.25 (m, 2H, CON$H$ & pyrrole H4), 6.34 (m, 1H, pyrrole H3), 7.14 (m, 1H, pyrrole H5); selected $^1$H NMR of minor isomer, δ 3.31 (s, 3H, $SO_2Me$); $^{13}$C NMR ($CDCl_3$, 75 MHz) of major isomer, δ 21.9, 22.7, 24.8, 30.6, 32.9, 41.4, 43.0, 50.8, 52.3, 64.8, 111.1, 112.1, 122.9, 137.0, 173.1, 173.6; selected $^{13}$C NMR of minor isomer, δ 30.7, 33.0, 41.5, 50.8, 65.1, 173.1, 173.5; HRMS calcd for $C_{16}H_{24}N_2O_5S$ (M-$H_2O$) 356.1406, found 356.1407.

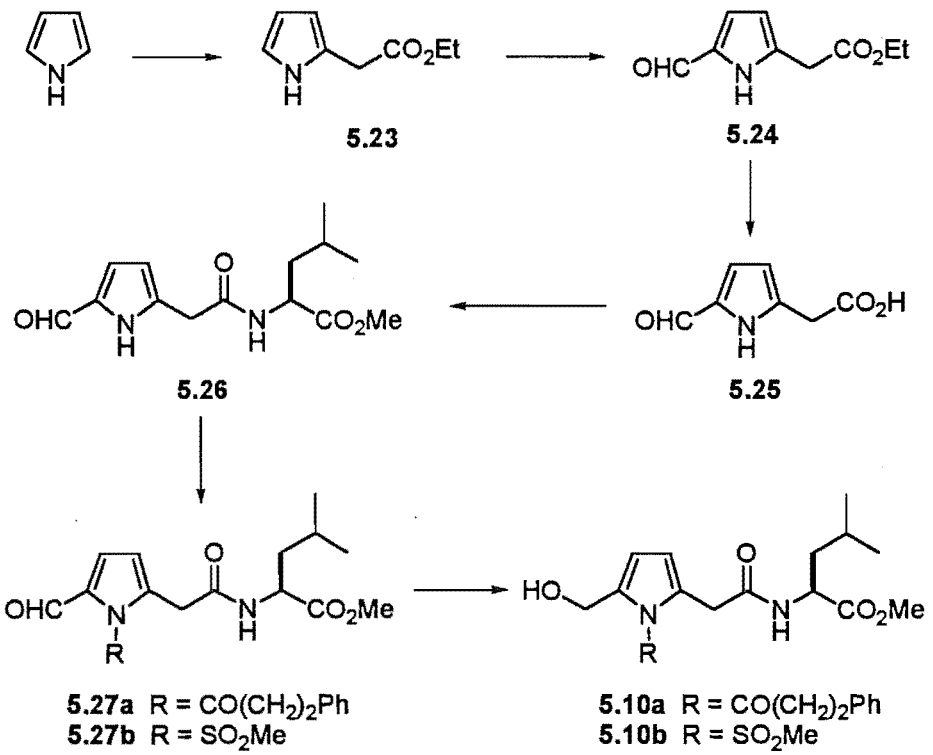
**_N_-{4-Hydroxy-4-[1-(phenylsulfonyl)pyrrol-2-yl]}butanoyl-_L_-leucine methyl ester 5.9b**

General procedure B was carried out using the pyrrole **5.21** (50 mg, 0.17 mmol) and phenylsulfonyl chloride (52 μL, 0.41 mmol, 2.4 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave an inseparable mixture of **5.21** and **5.22b** (**1** : **5** by $^1$H NMR) which was used in the next step without further purification. $^1$H NMR ($CDCl_3$, 300 MHz) of **5.22b** assigned from the mixture, δ 0.88 (m, 6H, CH$Me_2$), 1.42 – 1.62 (m, 3H, C$H_2$C$H$Me$_2$), 2.51 (m, 2H, C$H_2$CONH), 3.07 (m, 2H, COC$H_2$), 3.68 (s, 3H, $CO_2Me$), 4.52 (m, 1H, α$H$), 6.03 (d, $J$ = 8.3 Hz, 1H, CON$H$), 6.34 (m, 1H, pyrrole H4), 7.13 (m, 1H, pyrrole H3 ), 7.47 – 7.61 (m, 3H, phenyl Hs), 7.79 (m, 1H, pyrrole H5), 7.96 (m, 2H, phenyl Hs).

The above sample of _N_-phenylsulfonylpyrrole **5.22b** (48 mg, 0.11 mmol) was reduced with sodium borohydride by general procedure E. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:1) gave **5.9b** (28 mg, 38% overall for both steps), which was a mixture of diastereomers (**2** : **1** by $^1$H NMR), as a pale purple oil which solidified at 0 °C. An analytical sample was obtained by recrystallisation from ethyl

acetate/petroleum ether to give colourless crystals: mp 99 – 101 °C; IR ($CHCl_3$) 3568, 3433, 3317, 1740, 1670, 1514, 1367, 1177 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) of major isomer, δ 0.94 (d, $J$ = 5.9 Hz, 6H, $CHMe_2$), 1.51 – 1.67 (m, 3H, $CH_2CHMe_2$), 2.15 (m, 2H, $CHOHCH_2$), 2.42 (m, 2H, $CH_2CONH$), 3.75 (s, 3H, $CO_2Me$), 4.63 (m, 1H, αH), 4.96 (m, 1H, CHOH), 6.10 (d, $J$ = 7.3 Hz, 1H, CONH), 6.25 (m, 1H, pyrrole H4), 6.33 (m, 1H, pyrrole H3), 7.26 (m, 1H, pyrrole H5), 7.48 – 7.63 (m, 3H, phenyl Hs), 7.78 (m, 2H, phenyl Hs); selected $^1H$ NMR of minor isomer, δ 3.73 (s, 3H, $CO_2Me$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) of major isomer, δ 21.9, 22.7, 24.8, 31.0, 32.9, 41.6, 50.8, 52.3, 65.3, 112.0, 112.7, 123.4, 126.5, 129.5, 134.0, 138.0, 139.1, 172.9, 173.5; selected $^{13}C$ NMR of minor isomer, δ 31.0, 65.4, 126.5; HRMS calcd for $C_{21}H_{26}N_2O_5S$ (M-$H_2O$) 418.1562, found 418.1557. Anal. Calcd for $C_{21}H_{28}N_2O_6S$: C, 57.78; H, 6.47; N, 6.42. Found: C, 57.86; H, 6.25; N, 6.51.

### 8.5.2.3. Preparation of peptidomimetics of type **5.10**




**5.27a** R = $CO(CH_2)_2Ph$
**5.27b** R = $SO_2Me$

**5.10a** R = $CO(CH_2)_2Ph$
**5.10b** R = $SO_2Me$

**Ethyl 2-(1*H*-pyrrol-2-yl)ethanoate 5.23**

Ethylmagnesium bromide (20.0 mL of 3.9 M solution in ether, 78.4 mmol, 3.3 equiv) was added to a stirred solution of pyrrole (5.57 g, 83.1 mmol, 3.5 equiv) in THF (125 mL) cooled in an ice-sodium chloride bath (–10 °C bath temperature) under $N_2$. The resulting suspension was stirred at –10 °C for 30 min and then at rt for a further 30 min. The reaction temperature was re-cooled to –10 °C and ethyl bromoacetate (2.63 mL, 23.7 mmol) was added rapidly (small exotherm). The cooling bath was removed and the reaction mixture was stirred at rt for 2 h. Ether and excess saturated aqueous ammonium chloride were added. The organic phase was separated, washed with saturated aqueous ammonium chloride, dried and evaporated under reduced pressure. The residual oil was fractionally distilled *in vacuo* using a short path distillation head, to give first recovered pyrrole, followed by **5.23** (2.20 g, 61%) as a pale yellow oil: IR ($CHCl_3$) 3470, 1728 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 3.61 (s, 2H, $CH_2CO_2$), 4.14 (q, $J$ = 7.3 Hz, 2H, $CO_2CH_2CH_3$), 5.99 (m, 1H, pyrrole H4), 6.10 (m, 1H, pyrrole H3), 6.66 (m, 1H, pyrrole H5), 8.79 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 13.9, 33.0, 60.8, 107.0, 107.9, 117.5, 123.1, 171.1; HRMS calcd for $C_8H_{11}NO_2$ 153.0790, found 153.0788.

**Ethyl 2-(5-formyl-1*H*-pyrrol-2-yl)ethanoate 5.24**

General procedure A was carried out using pyrrole **5.23** (2.19 g, 14.3 mmol) and the resulting oil was distilled at reduced pressure to give **5.24** (1.45 g, 56%) as a pale orange solid. An analytical sample was obtained by sublimation under reduced pressure to give a white crystalline solid: mp 73 – 75 °C; IR ($CHCl_3$) 3433, 1732, 1651 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.29 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 3.72 (s, 2H, $CH_2CO_2$), 4.22 (q, $J$ = 7.3 Hz, 2H, $CO_2CH_2CH_3$), 6.18 (m, 1H, pyrrole H3), 6.90 (m, 1H, pyrrole H4), 9.45 (s, 1H, C*H*O), 9.88 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.0, 33.4, 61.4, 111.0, 122.2, 132.6, 133.8, 169.6, 178.7; HRMS calcd for $C_9H_{11}NO_3$ 181.0739, found 181.0738. Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.89; H, 5.99, N, 7.71.

**2-(5-Formyl-1*H*-pyrrol-2-yl)ethanoic acid 5.25**

The pyrrole ester **5.24** (300 mg, 1.66 mmol) was hydrolysed with sodium hydroxide by general procedure J to give **5.25** (213 mg, 84%) as a yellow solid: mp 123 – 124 °C; IR ($CHCl_3$) 3433, 3263, 1726, 1651 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 300 MHz) δ 3.86 (s, 2H, $CH_2CO_2$), 6.33 (dd, $J$ = 3.4 & 2.4 Hz, 1H, pyrrole H3), 7.08 (dd, $J$ = 3.4 & 2.4 Hz,, 1H, pyrrole H4), 9.43 (s, 1H, CHO), 11.49 (bs, 1H, NH); $^{13}C$ NMR (acetone-$d_6$, 75 MHz) δ 33.0, 111.2, 122.6, 132.4, 135.3, 171.9, 179.3; HRMS calcd for $C_7H_7NO_3$ 153.0426, found 153.0427.

***N*-[2-(5-Formyl-1*H*-pyrrol-2-yl)]ethanoyl-*L*-leucine methyl ester 5.26**

The pyrrole acid **5.25** (210 mg, 1.37 mmol) was coupled with *L*-leucine methyl ester hydrochloride (274 mg, 1.51 mmol, 1.1 equiv) according to general procedure L. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:1) gave **5.26** (326 mg, 85%) as a pale orange solid: mp 54 °C; IR ($CHCl_3$) 3422, 3298, 1744, 1676, 1653, 1518, 1491 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (d, $J$ = 5.9 Hz, 6H, $CHMe_2$), 1.48 – 1.64 (m, 3H, $CH_2CHMe_2$), 3.69 (s, 2H, $CH_2CO_2$), 3.72 (s, 3H, $CO_2Me$), 4.63 (m, 1H, αH), 6.24 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 6.79 (d, $J$ = 7.8 Hz, 1H, CONH), 6.95 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 9.38 (s, 1H, CHO), 10.89 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.6, 22.5, 24.7, 35.4, 40.9, 50.9, 52.2, 111.2, 122.9, 132.4, 135.5, 168.8, 173.6, 178.7; HRMS calcd for $C_{14}H_{20}N_2O_4$ 280.1423, found 280.1430; Anal. Calcd for $C_{14}H_{20}N_2O_4$: C, 59.99; H, 7.19; N, 9.99. Found: C, 60.16; H, 7.20; N, 9.84.

The pyrrole acid **5.25** (120 mg, 0.78 mmol) was also coupled with *L*-leucine methyl ester hydrochloride (142 mg, 0.78 mmol, 1 equiv) according to general procedure K. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.26** (178 mg, 81%) as a pale orange solid. Spectral data as recorded above.

***N*-{2-[5-Formyl-1-(3-phenylpropionyl)pyrrol-2-yl]}ethanoyl-*L*-leucine methyl ester 5.27a**

A solution of pyrrole **5.26** (60 mg, 0.21 mmol), DMAP (3 mg, 0.02 mmol, 0.1 equiv) and Hünig's base (41 μL, 0.24 mmol, 1.1 equiv) in dichloromethane (8 mL) was cooled in an ice-sodium chloride bath (–10 °C bath temperature) under $N_2$ and hydrocinnamoyl chloride (35 μL, 0.24 mmol, 1.1 equiv), dissolved in dichloromethane (2

mL), was added. The resultant mixture was stirred at –10 °C for 30 min and then at rt for 24 h, before being worked up by the method described in general procedure C. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.27a** (72 mg, 81%) as a yellow solid. An analytical sample was obtained by recrystallisation from petroleum ether to give small white crystals: mp 71 °C; IR ($CHCl_3$) 3427, 1740, 1666 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, $J$ = 5.9 Hz, 3H, CH$Me_2$), 0.91 (d, $J$ = 5.9 Hz, 3H, CH$Me_2$), 1.50 – 1.66 (m, 3H, C$H_2$C$H$Me$_2$), 3.07 (m, 2H, C$H_2$Ph), 3.24 (m, 2H, COC$H_2$), 3.56 (ABq, $J$ = 16.1 Hz, 1H, C$H_2$CO), 3.62 (ABq, $J$ = 16.1 Hz, 1H, C$H_2$CO), 3.70 (s, 3H, $CO_2Me$), 4.56 (m, 1H, α$H$), 6.27 (d, $J$ = 3.9 Hz, 1H, pyrrole H3), 6.42 (d, $J$ = 7.8 Hz, 1H, CON$H$), 7.04 (d, $J$ = 3.9 Hz, 1H, pyrrole H4), 7.16 – 7.29 (m, 5H, phenyl Hs), 9.49 (s, 1H, C$H$O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.9, 22.7, 24.8, 31.1, 35.2, 41.4, 41.7, 51.0, 52.3, 113.2, 126.3, 127.4, 128.3, 128.5, 133.7, 137.6, 139.9, 167.8, 173.1, 176.7, 178.1; HRMS calcd for $C_{23}H_{28}N_2O_5$ 412.1998, found 412.1995.

**$N$-{2-[5-Formyl-1-(methanesulfonyl)pyrrol-2-yl]}ethanoyl-$L$-leucine methyl ester 5.27b**

The modified general procedure B was carried out using pyrrole **5.26** (50 mg, 0.18 mmol) and methanesulfonyl chloride (17 μL, 0.21 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.27b** (45 mg, 70%) as a pale yellow oil: IR ($CHCl_3$) 3429, 3327, 1740, 1684, 1518, 1491, 1369, 1180 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.95 (d, $J$ = 6.3 Hz, 6H, CH$Me_2$), 1.53 – 1.80 (m, 3H, C$H_2$C$H$Me$_2$), 3.65 (s, 3H, $SO_2Me$), 3.74 (s, 3H, $CO_2Me$), 3.93 (ABq, $J$ = 16.6 Hz, 1H, C$H_2$CO), 4.00 (ABq, $J$ = 16.6 Hz, 1H, C$H_2$CO), 4.64 (m, 1H, α$H$), 6.19 (d, $J$ = 8.3 Hz, 1H, CON$H$), 6.25 (d, $J$ = 3.9 Hz, 1H, pyrrole H3), 7.12 (d, $J$ = 3.9 Hz, 1H, pyrrole H4), 9.70 (s, 1H, C$H$O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.8, 22.8, 24.6, 36.2, 41.6, 42.4, 50.9, 52.4, 115.1, 126.7, 135.4, 138.6, 168.6, 173.3, 178.2; HRMS calcd for $C_{15}H_{22}N_2O_6S$ 358.1199, found 358.1190.

An analogous reaction using the pyrrole **5.26** (typically 60 mg, 0.21 mmol) and either phenylsulfonyl chloride, β-*trans*-styrenesulfonyl chloride or 2-phenylethanesulfonyl chloride proved unsuccessful. $^1H$ NMR spectra of the crude reaction work ups did not indicate the presence of any of the desired $N$-substituted products.

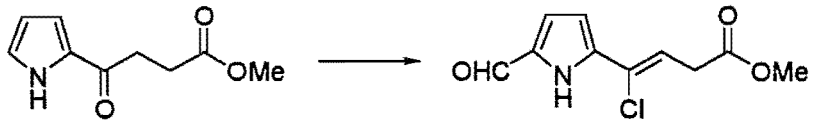
**_N_-{2-[5-Hydroxymethyl-1-(3-phenylpropionyl)pyrrol-2-yl]}ethanoyl-_L_-leucine methyl ester 5.10a**

The _N_-hydrocinnamoylpyrrole **5.27a** (25 mg, 0.06 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.10a** (11 mg, 44%) as an unstable orange oil which slowly decomposed at 0 °C. $^1$H NMR showed the compound to be *ca.* 90% pure: IR ($CHCl_3$) 1738, 1674, 1514 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.89 (d, $J$ = 5.9 Hz, 6H, CH*Me*$_2$), 1.44 – 1.62 (m, 3H, C*H*$_2$C*H*Me$_2$), 3.01 – 3.10 (m, 3H, O*H* & C*H*$_2$Ph), 3.30 (m, 2H, COC*H*$_2$), 3.68 (s, 3H, CO$_2$*Me*), 3.74 (s, 2H, C*H*$_2$CO), 4.54 – 4.62 (m, 3H, C*H*$_2$OH & α*H*), 6.03 (d, $J$ = 7.8 Hz, 1H, CON*H*), 6.12 (d, $J$ = 3.4 Hz, 1H, pyrrole H), 6.18 (d, $J$ = 3.4 Hz, 1H, pyrrole H), 7.18 – 7.32 (m, 5H, phenyl Hs); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 21.8, 22.7, 24.8, 30.6, 37.8, 39.1, 41.2, 50.8, 52.3, 58.5, 113.8, 114.9, 126.4, 128.3, 128.4, 128.5, 136.0, 140.0, 169.3, 173.2, 174.5; HRMS (FAB) calcd for $C_{23}H_{31}N_2O_5$ (MH) 415.2233, found 415.2230.

**_N_-{2-[5-Hydroxymethyl-1-(methanesulfonyl)pyrrol-2-yl]}ethanoyl-_L_-leucine methyl ester 5.10b**

The _N_-mesylpyrrole **5.27b** (35 mg, 0.10 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:1) gave **5.10b** (29 mg, 83%) as an orange solid. An analytical sample was obtained by recrystallisation from methanol to give thin colourless needles: mp 150 – 151 °C; IR ($CHCl_3$) 1740, 1676, 1364, 1169 cm$^{-1}$; $^1$H NMR ($CD_3OD$, 300 MHz) δ 0.94 (m, 6H, CH*Me*$_2$), 1.55 – 1.81 (m, 3H, C*H*$_2$C*H*Me$_2$), 3.40 (s, 3H, SO$_2$*Me*), 3.70 (s, 3H, CO$_2$*Me*), 3.80 (ABq, $J$ = 17.1 Hz, 1H, C*H*$_2$CO), 3.89 (ABq, $J$ = 17.1 Hz, 1H, C*H*$_2$CO), 4.47 (m, 1H, α*H*), 4.67 (ABq, $J$ = 13.2 Hz, 1H, C*H*$_2$OH), 4.74 (ABq, $J$ = 13.2 Hz, 1H, C*H*$_2$OH), 6.09 (d, $J$ = 3.4 Hz, 1H, pyrrole H3), 6.22 (d, $J$ = 3.4 Hz, 1H, pyrrole H4), 8.30 (d, $J$ = 7.8 Hz, 1H, CON*H*); $^{13}$C NMR ($CD_3OD$, 75 MHz) δ 22.1, 23.6, 26.1, 36.7, 41.8, 42.8, 52.6, 53.0, 58.4, 114.7, 115.1, 132.3, 137.5, 173.2, 175.1; HRMS calcd for $C_{15}H_{24}N_2O_6S$ 360.1355, found 360.1366. Anal. Calcd for $C_{15}H_{24}N_2O_6S$: C, 49.99; H, 6.71; N, 7.77. Found: C, 49.70; H, 6.93; N, 7.50.

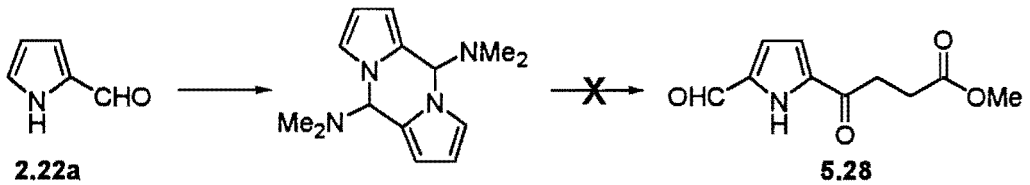
### 8.5.2.4. Attempted preparation of peptidomimetics of type **5.11**




**5.18** **5.30**

**Methyl (*Z*)-4-chloro-4-(5-formyl-1*H*-pyrrol-2-yl)-3-butenoate 5.30**

General procedure A was carried out using pyrrole **5.18** (1.00 g, 5.52 mmol). Distillation of the residue at reduced pressure followed by flash chromatography on silica (ethyl acetate/petroleum ether, 1:3) and recrystallisation from petroleum ether gave **5.30** (168 mg, 13%) as white needles: mp 80 °C; IR ($CHCl_3$) 3433, 1738, 1651 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.47 (d, $J$ = 6.8 Hz, 2H, $CHCH_2$), 3.75 (s, 3H, $CO_2Me$), 6.57 (m, 1H, pyrrole H3), 6.67 (t, $J$ = 6.8 Hz, 1H, $CHCH_2$), 6.94(m, 1H, pyrrole H4), 9.52 (s, 1H, CHO), 10.29 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 34.3, 52.2, 110.4, 119.9, 121.8, 125.5, 133.0, 136.1, 170.7, 179.3; HRMS calcd for $C_{10}H_{10}ClNO_3$ 227.0349, found 227.0347.




**2.22a** **5.31** **5.28**

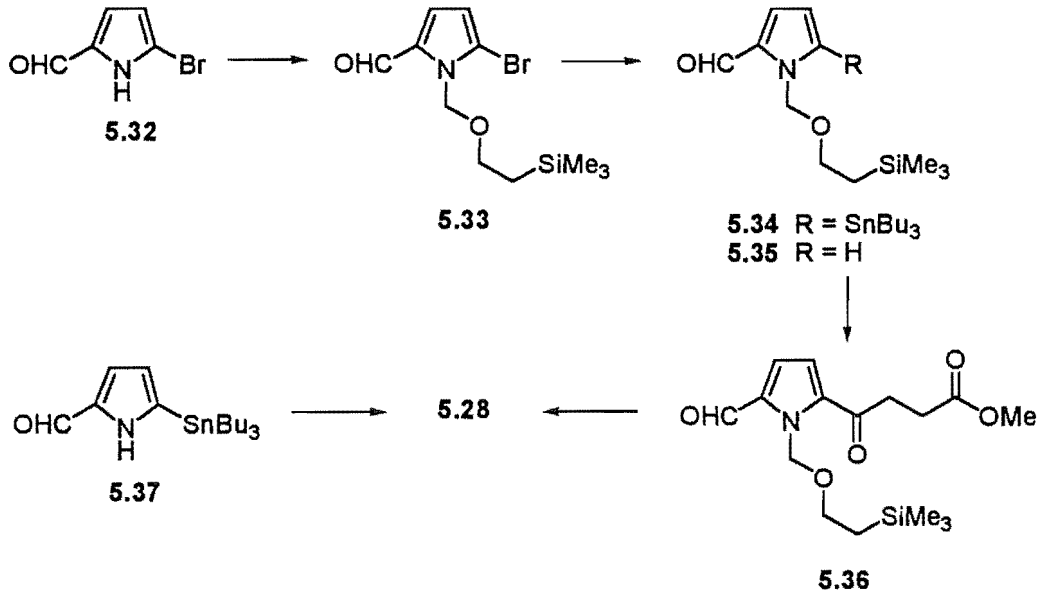
**5,10-Bisdimethylamino-5*H*,10*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine 5.31**

A solution of pyrrole-2-carboxaldehyde **2.22a** (4.00g, 42.1 mmol) in 40% aqueous dimethylamine (20 mL) was stirred at rt for 3½ h and then diluted with an equal volume of water. The solid was collected by filtration, washed successively with 1 M aqueous sodium hydroxide and ethyl acetate, then dried under high vacuum (oil pump) to give **5.31** (3.91 g, 76%) as a white solid: mp 110 – 111 °C (lit.[29] 113 °C); IR ($CHCl_3$) 1655, 1308 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.21 (s, 12H, $NMe_2$), 5.87 (s, 2H, H-5,10), 6.17 (m, 2H, H-1,6), 6.26 (m, 2H, H-2,7), 6.95 (m, 2H, H-3,8); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 39.1, 71.9, 105.5, 108.3, 119.5, 125.3; HRMS calcd for $C_{14}H_{20}N_4$ 244.1688, found 244.1689.

**Attempted preparation of the acylpyrrole 5.28 by anion chemistry**

Methyl succinyl chloride (343 μL, 2.79 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (299 mg, 3.07 mmol, 1.1 equiv) were dissolved in dichloromethane (10 mL) at rt under $N_2$. The mixture was cooled to 0 °C and pyridine (496 μL, 6.14 mmol, 2.2 equiv) was added. The resulting mixture was then stirred at rt for 1 h. The solution was diluted with saturated aqueous ammonium chloride (10 mL) and ether (15 mL) was added. The organic phase was separated, washed with saturated aqueous sodium hydrogencarbonate (10 mL), saturated aqueous ammonium chloride (10 mL), saturated aqueous brine (10 mL), dried and evaporated to give 3-carbomethoxypropionyl-*N*-methoxy-*N*-methylamide (441 mg, 90%) as a yellow oil which was used in the next step without purification. $^1$H NMR ($CDCl_3$, 300 MHz) δ 2.65 (m, 2H), 2.77 (m, 2H), 3.19 (s,3H), 3.70 (s,3H), 3.73 (s, 3H).

The azafulvene dimer **5.31** (200 mg, 0.82 mmol) was dissolved in THF (10 mL) under $N_2$ at –15 °C and *tert*-butyl lithium (1.36 mL of 1.5 M solution in pentane, 2.05 mmol, 2.5 equiv.) was added over 2 min. The resulting solution was stirred at –15 °C for 15 min and then at 0 °C for 30 min to give a deep violet solution. The reaction mixture was cooled to –78 °C and 3-carbomethoxypropionyl-*N*-methoxy-*N*-methylamide (430 mg, 2.46 mmol, 3 equiv) dissolved in THF (2 mL) was added. The resulting mixture was stirred at –78 °C for 1 h and then at rt for 2 h. A solution of sodium acetate (302 mg, 3.68 mmol, 4.5 equiv) in water (10 mL) was added and the mixture was heated at reflux for 4 h. The reaction mixture was then poured into dilute aqueous sodium hydrogencarbonate, and extracted with dichloromethane (3x10 mL). The combined organic extracts were washed with saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography of the resulting oil on silica (ethyl acetate/petroleum ether, 1:2) gave only the hydrolysed starting material **2.22a** (106 mg, 68%).




**5-Bromo-1*H*-pyrrole-2-carboxaldehyde 5.32**

5-Bromo-1*H*-pyrrole-2-carboxaldehyde **5.32** was prepared by the indirect method described by Bray and Muchowski.[30] Spectral data was consistent with that reported by Bray and Muchowski: mp 92 – 94 °C (lit.[30] mp 94 – 96 °C); IR ($CS_2$) 3427, 3215, 1665, 1655 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 6.33 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.94 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 9.37 (s, 1H, C*H*O), 11.17 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 112.1, 113.8, 123.3, 133.7, 178.3; HRMS calcd for $C_5H_4BrNO$ 172.9476, found 172.9479.

**5-Bromo-1-[2-(trimethylsilyl)ethoxymethyl]pyrrole-2-carboxaldehyde 5.33**

The modified general procedure B was carried out with the bromopyrrole **5.32** (288 mg, 1.66 mmol) and 2-(trimethylsilyl)ethoxymethyl chloride (322 μL, 1.82 mmol, 1.1 equiv) using DMF as the solvent. The mixture was then poured into ice-cold 10% aqueous sodium hydrogencarbonate solution (10 mL), and the product was extracted into ether (3x10 mL). The combined ethereal phases were washed with saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:10) gave **5.33** as a pale yellow oil (447 mg, 89%): IR ($CS_2$) 1670, 1099 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ –0.07 (s, 9H, Si*Me*$_3$), 0.87 (t, $J$ = 8.1 Hz, 2H, C*H*$_2$Si), 3.56 (t, $J$ = 8.1 Hz, 2H, OC*H*$_2$CH$_2$), 5.77 (s, 2H, C*H*$_2$O), 6.35 (m, 1H, pyrrole H4), 6.92 (m, 1H, pyrrole H3), 9.40 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75

MHz) δ –1.5, 17.8, 66.0, 74.2, 110.6, 113.8, 116.7, 125.4, 178.2; HRMS calcd for $C_{11}H_{18}BrNO_2Si$ 303.0290, found 303.0293.

**5-(tri-*n*-Butylstannyl)-1-[2-(trimethylsilyl)ethoxymethyl]pyrrole-2-carboxaldehyde 5.34**

To a solution of morpholine (69 μL, 0.80 mmol, 1.1 equiv) in THF (6 mL) at –78 °C under $N_2$ was added *n*-butyl lithium (497 μL of 1.6 M solution in hexane, 0.80 mmol, 1.1 equiv). After stirring for 20 min, **5.33** (220 mg, 0.72 mmol) dissolved in THF (2 mL) was slowly added. After stirring for another 20 min, *tert*-butyl lithium (936 μL of 1.7 M solution in pentane, 1.59 mmol, 2.2 equiv) was added dropwise. Stirring was continued at –78 °C for a further 15 min before tri-*n*-butylstannyl chloride (216 μL, 0.80 mmol, 1.1 equiv) was added. The reaction temperature was left to warm to rt during 1 h, and was stirred at rt for 30 min. The solution was then poured into water and was extracted with ether (3x10 mL). The combined ethereal phase was washed with saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:20) gave two compounds. The first to elute was **5.34** (222 mg, 60%) as a pale yellow oil: IR ($CHCl_3$) 1655, 1088 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ –0.04 (s, 9H, Si*Me*$_3$), 0.86 – 1.63 (m, 29H, Sn*Bu*$_3$ & C*H*$_2$Si), 3.47 (m, 2H, OC*H*$_2$CH$_2$), 5.74 (s, 2H, C*H*$_2$O), 6.37 (m, 1H, pyrrole H4), 6.97 (m, 1H, pyrrole H3), 9.53 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ –1.5, 10.4, 13.6, 17.8, 27.3, 29.0, 65.3, 76.3, 121.2, 125.9, 135.7, 148.8, 178.4; HRMS calcd for $C_{19}H_{36}NO_2SiSn$ (M-Bu) 458.1537, found 458.1541. The second compound to elute was the debrominated starting material **5.35** (41 mg, 25%) as a colourless oil: IR ($CHCl_3$) 1665, 1081 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ –0.06 (s, 9H, Si*Me*$_3$), 0.88 (t, *J* = 8.3 Hz, 2H, C*H*$_2$Si), 3.52 (t, *J* = 8.3 Hz, 2H, OC*H*$_2$CH$_2$), 5.69 (s, 2H, C*H*$_2$O), 6.28 (m, 1H, pyrrole H4), 6.96 (m, 1H, pyrrole H3), 7.13 (m, 1H, pyrrole H5), 9.57 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ –1.5, 17.8, 66.1, 76.5, 110.6, 110.5, 125.2, 131.0, 131.8, 179.6; HRMS calcd for $C_{11}H_{19}NO_2Si$ 225.1185, found 225.1180.

**Methyl 4-oxo-4-{5-formyl-1-[2-(trimethylsilyl)ethoxymethyl]pyrrol-2-yl}butanoate 5.36**

The stannylpyrrole **5.34** (215 mg, 0.42 mmol) was coupled with methyl succinyl chloride (51 μL, 0.42 mmol, 1 equiv) according to general procedure M with the exception that the resulting residue was not treated with potassium fluoride. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **5.36** (105 mg, 74%) as an orange oil: IR ($CHCl_3$) 1734, 1692, 1666 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ –0.10 (s, 9H, Si*Me*$_3$), 0.83 (t, *J* = 7.8 Hz, 2H, C*H*$_2$Si), 2.70 (t, *J* = 6.8 Hz, 2H, C*H*$_2$CO$_2$Me), 3.20 (t, *J* = 6.8 Hz, 2H, COC*H*$_2$), 3.50 (t, *J* = 7.8 Hz, 2H, OC*H*$_2$CH$_2$), 3.67 (s, 3H, CO$_2$*Me*), 6.12 (s, 2H, C*H*$_2$O), 6.95 (d, *J* = 4.4 Hz, 1H, pyrrole H3), 7.04 (d, *J* = 4.4 Hz, 1H, pyrrole H4), 9.83 (s, 1H, C*H*O); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ –1.6, 17.8, 27.7, 35.1, 51.8, 66.0, 73.9, 118.4, 121.7, 135.2, 136.0, 173.0, 181.6, 190.8; HRMS calcd for $C_{14}H_{21}NO_5Si$ (M-$C_2H_4$) 311.1189, found 311.1188.

**5-(tri-*n*-Butylstannyl)-1*H*-pyrrole-2-carboxaldehyde 5.37**

To a stirred solution of the azafulvene dimer **5.31** (1.00 g, 4.09 mmol) in THF (50 mL) cooled to –15 °C was added dropwise *tert*-butyl lithium (5.30 mL of 1.7 M solution in pentane, 9.00 mmol, 2.2 equiv). The resulting mixture was stirred for 15 min at –15 °C and then for 30 min at 0 °C. The violet solution formed was cooled to –78 °C and tri-*n*-butylstannyl chloride (2.44 mL, 9.00 mmol, 2.2 equiv) was slowly added. The reaction mixture was then stirred for 3 h at rt, before being hydrolysed with excess 0.4 M aqueous sodium acetate solution. The resulting mixture was extracted with dichloromethane, dried and concentrated under reduced pressure. The residue was dissolved in THF (30 mL) and a solution of sodium acetate (1.01 g, 12.28 mmol, 3 equiv) in water (30 mL) was added, and the resultant mixture was stirred at reflux temperature for 5 days. After cooling to rt the reaction mixture was extracted with dichloromethane (3x30 mL), and the combined organic phase was washed with water (2x50 mL), saturated aqueous brine (50 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (petroleum ether to ethyl acetate/petroleum ether, 1:10) gave **5.37** (1.18 g, 37% for 2 steps) as a pale yellow oil: IR ($CHCl_3$) 3445, 3285, 1645 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.86 – 1.63 (m, 27H, Sn*Bu*$_3$), 6.42 (dd, *J* = 3.9 & 2.4 Hz, 1H, pyrrole H4), 7.04 (dd, *J* = 3.9 & 2.4 Hz, 1H, pyrrole H3), 9.49 (s, 1H, C*H*O), 10.03 (bs, 1H, N*H*); $^{13}C$ NMR

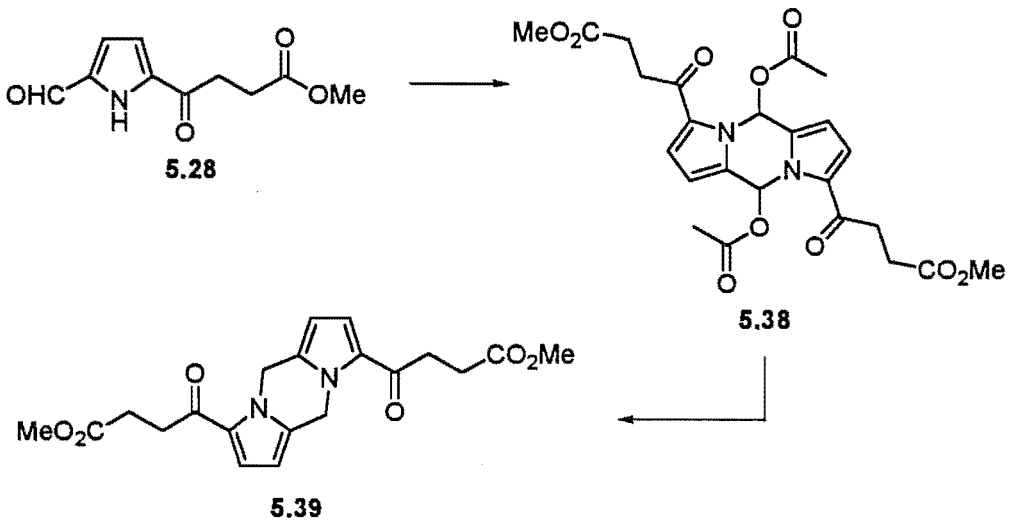
($CDCl_3$, 75 MHz) δ 10.0, 13.6, 27.2, 28.9, 121.0, 121.7, 136.6, 142.4, 178.0; HRMS calcd for $C_{13}H_{22}NOSn$ (M-Bu) 328.0723, found 328.0727.

**Methyl 4-oxo-4-(5-formyl-1*H*-pyrrol-2-yl)butanoate 5.28**

**Method A:** To a stirred solution of **5.36** (23 mg, 0.07 mmol) in dichloromethane (2 mL) cooled to 0 °C was slowly added boron trifluoride diethyl etherate (43 μL, 0.34 mmol, 5 equiv). The solution was left to come to rt after which time stirring was continued for 20 min. The reaction mixture was poured into dichloromethane (10 mL), washed with water (10 mL), saturated aqueous brine (10mL), dried and evaporated under reduced pressure. The resulting residue was dissolved in dichloromethane (2 mL) and a solution of sodium acetate (56 mg, 0.68 mmol, 10 equiv) in water (1 mL) was added. The stirred mixture was heated at reflux for 1 h and then worked up as described above. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.28** (11 mg, 75%) as a white solid. An analytical sample was obtained by recrystallisation from ethyl acetate/petroleum ether to give colourless crystals: mp 102 – 104 °C; IR ($CHCl_3$) 3422, 1736, 1682, 1665 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.78 (t, $J$ = 6.8 Hz, 2H, $CH_2CO_2Me$), 3.21 (t, $J$ = 6.8 Hz, 2H, $COCH_2$), 3.70 (s, 3H, $CO_2Me$), 6.97 (m, 2H, pyrrole H3 & pyrrole H4), 9.73 (s, 1H, CHO), 10.12 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 27.6, 33.3, 51.9, 115.5, 119.5, 134.8, 134.9, 173.0, 180.8, 189.8; HRMS calcd for $C_{10}H_{11}NO_4$ 209.0688, found 209.0685. Anal. Calcd for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.38; H, 5.17; N, 6.68.

**Method B:** The stannylpyrrole **5.37** (150 mg, 0.39 mmol) was coupled with methyl succinyl chloride (48 μL, 0.39 mmol, 1 equiv) according to general procedure M. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.28** (64 mg, 78%) as a white solid. Spectral data as recorded above.

**Attempted *N*-acylation of pyrrole 5.28 using general procedure B: Azafulvene dimer formation**




**3,8-Bis(3-carbomethoxy-1-oxopropyl)-5,10-bisacetyloxy-5*H*,10*H*-dipyrrolo[1,2-a: 1',2'-d]pyrazine 5.38**

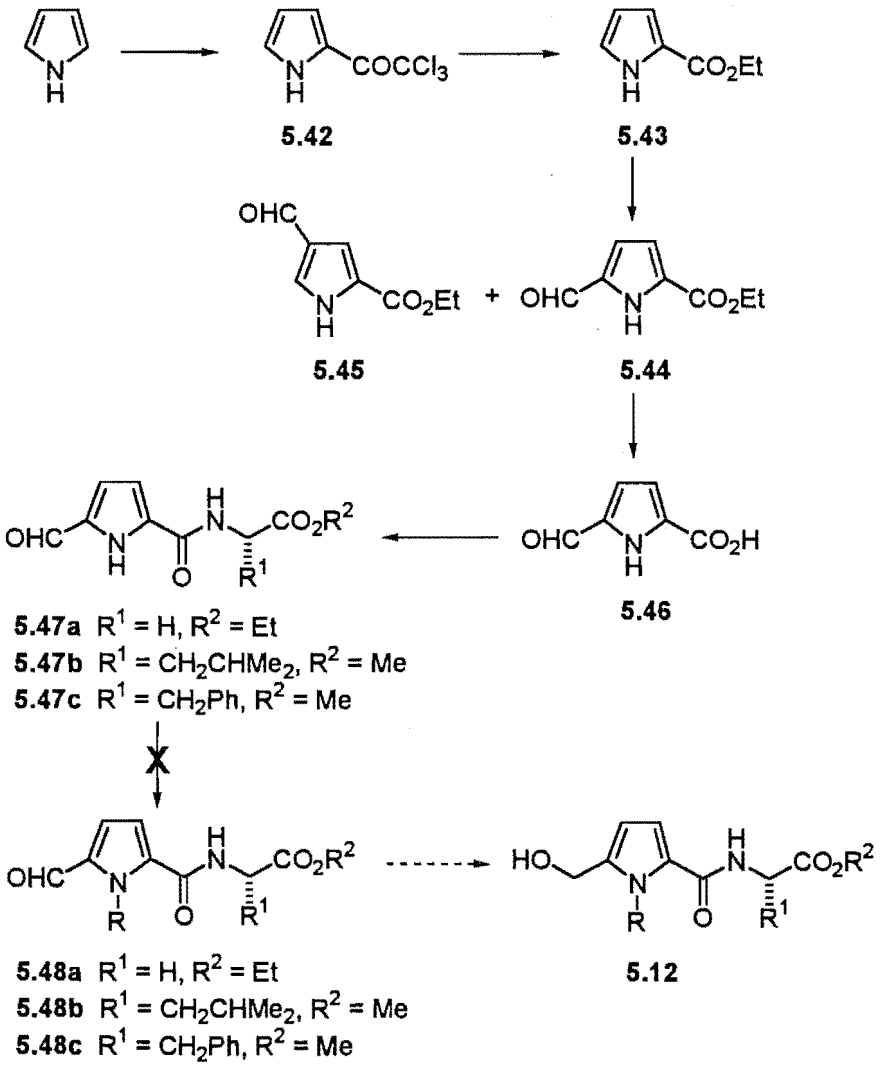
General procedure B was carried out using pyrrole **5.28** (20 mg, 0.10 mmol) and acetyl chloride (8 μL, 0.11 mmol, 1.2 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.38** (21 mg, 89%) as a pale yellow solid. An analytical sample was recrystallised from ethyl acetate/petroleum ether to give colourless crystals: mp 152 – 153 °C; IR ($CHCl_3$) 1738, 1666, 1121 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.06 (s, 6H, CO*Me*), 2.74 (t, *J* = 6.8 Hz, 4H, $CH_2CO_2Me$), 3.17 (m, 4H, $COCH_2$), 3.69 (s, 6H, $CO_2Me$), 6.65 (d, *J* = 4.4 Hz, 2H, H-1,6), 7.11 (d, *J* = 4.4 Hz, 2H, H-2,7), 8.41 (s, 2H, H-5,10); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 20.8, 27.7, 33.6, 51.8, 71.6, 111.7, 119.9, 129.6, 131.9, 169.4, 173.1, 188.9; HRMS (FAB) calcd for $C_{22}H_{23}N_2O_8$ (MH–AcOH) 443.1454, found 443.1453.

**3,8-Bis(3-carbomethoxy-1-oxopropyl)-5*H*,10*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine 5.39**

The azafulvene dimer **5.38** (20 mg, 0.04 mmol) was dissolved in ether (10 mL) at 0 °C under $N_2$. Zinc borohydride (284 μL of 0.14 M solution in ether, 0.04 mmol, 1 equiv.) and trimethylsilyl chloride (11 μL, 0.09 mmol, 2.2 equiv.) were added and the resultant solution was stirred at 0 °C for 2 h. Water (2 mL) and then 10% aqueous glacial acetic acid (2 mL) were carefully added to quench the reaction. The separated aqueous

phase was extracted with dichloromethane (2x10 mL) and the combined organic phase was washed with water (2x10 mL), saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.39** (2 mg, 16%) as a white solid: mp 165 °C; IR ($CHCl_3$) 1734, 1649 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.72 (t, $J$ = 6.8 Hz, 4H, $CH_2CO_2Me$), 3.16 (t, $J$ = 6.8 Hz, 4H, $COCH_2$), 3.71 (s, 6H, $CO_2Me$), 5.67 (s, 4H, H-5,10), 6.15 (d, $J$ = 3.9 Hz, 2H, H-1,6), 7.09 (d, $J$ = 3.9 Hz, 2H, H-2,7); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 28.2, 33.3, 43.5, 51.8, 105.8, 119.7, 129.0, 132.4, 173.5, 188.5; HRMS (FAB) calcd for $C_{20}H_{23}N_2O_6$ (MH) 387.1556, found 387.1568.

### 8.5.2.5. Attempted preparation of peptidomimetics of type **5.12**




**5.42** **5.43** **5.45** **5.44** **5.46**

**5.47a** $R^1$ = H, $R^2$ = Et
**5.47b** $R^1$ = $CH_2CHMe_2$, $R^2$ = Me
**5.47c** $R^1$ = $CH_2Ph$, $R^2$ = Me

**5.48a** $R^1$ = H, $R^2$ = Et
**5.48b** $R^1$ = $CH_2CHMe_2$, $R^2$ = Me
**5.48c** $R^1$ = $CH_2Ph$, $R^2$ = Me

**5.12**

**2-(Trichloroacetyl)-1*H*-pyrrole 5.42**

To a solution of trichloroacetyl chloride (7.32 mL, 66 mmol, 1.1 equiv) in ether (10 mL) was added a solution of pyrrole (4.14 mL, 60 mmol) in ether (33 mL) via a dropping funnel over a 3 h period. The exothermic reaction caused the mixture to reflux during the addition. The resulting mixture was stirred for 1 h before the reaction was quenched slowly with the addition of a solution of potassium carbonate (5.2 g) in water (20 mL). The layers were separated, and the organic phase was dried, treated with activated charcoal (300 mg), and filtered. The solvent was removed under reduced pressure, and the residue was dissolved in petroleum ether (~12 mL). The solution was cooled in ice, and the title product crystallised out as a tan solid. The solid was collected by filtration, washed with cold petroleum ether (10 mL) and air dried to give **5.42** (10.29 g, 81%): mp 72 – 74 °C (lit.[31] 73 – 75 °C); IR ($CHCl_3$) 3444, 1665 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 6.39 (m, 1H, pyrrole H4), 7.19 (m, 1H, pyrrole H3), 7.41 (m, 1H, pyrrole H5), 9.87 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 94.9, 111.8, 121.4, 122.9, 127.4, 173.3; HRMS calcd for $C_6H_4Cl_3NO$ 210.9359, found 210.9359.

**Ethyl 1*H*-pyrrole-2-carboxylate 5.43**

To a 100 mL RBF was added absolute ethanol (20 mL) and sodium metal (~150 mg). Once all the sodium had dissolved, **5.42** (10.29 g, 48 mmol) was added portionwise over a 10 min period. After the addition was complete, the solution was stirred for 30 min, and evaporated to dryness under reduced pressure. The oily residue was then partitioned between ether and 3 M aqueous hydrochloric acid. The ether layer was separated, and the aqueous layer was extracted once with ether. The ether solutions were combined, washed once with saturated aqueous sodium hydrogencarbonate, dried and evaporated under reduced pressure. The residue was distilled at reduced pressure to give **5.43** (6.58 g, 98%) as a pale yellow oil which crystallised upon standing: mp 39 – 40 °C (lit.[31] 40 – 42 °C); IR ($CHCl_3$) 3459, 3313, 1700 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.35 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 4.33 (q, $J$ = 7.3 Hz, 2H, $CO_2CH_2CH_3$), 6.25 (m, 1H, pyrrole H4), 6.92 – 6.96 (m, 2H, pyrrole H3 & pyrrole H5), 9.82 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.3, 60.3, 110.2, 115.2, 122.8, 123.0, 161.5; HRMS calcd for $C_7H_9NO_2$ 139.0633, found 139.0633.

**Ethyl 5-formyl-1*H*-pyrrole-2-carboxylate 5.44**

General procedure A was carried out using the pyrrole **5.43** (6.58 g, 47 mmol). Distillation of the resulting residue at reduced pressure, followed by flash chromatography on silica (ethyl acetate/petroleum ether, 1:3 to 1:1) gave two compounds. The first to elute was **5.44** (5.29 g, 67%) as a pink solid. An analytical sample was obtained by sublimation under reduced pressure to give a white crystalline solid: mp 72 – 74 °C (lit.[32] 75 °C); IR ($CHCl_3$) 3428, 1713, 1669 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.39 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 4.41 (q, $J$ = 7.3 Hz, 2H, $CO_2CH_2CH_3$), 6.93 – 6.97 (m, 2H, pyrrole H3 & pyrrole H4), 9.70 (s, 1H, C*H*O), 10.69 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 61.2, 115.4, 119.6, 128.6, 134.5, 160.4, 180.5; HRMS calcd for $C_8H_9NO_3$ 167.0582, found 167.0583. Anal. Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.35; H, 5.47; N, 8.31. The second compound to elute was **5.45** (1.21 g, 15%) as a white solid: mp 102 – 104 °C (lit.[32] 104 – 106 °C); IR ($CHCl_3$) 3437, 1707, 1678 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.38 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 4.37 (q, $J$ = 7.3 Hz, 2H, $CO_2CH_2CH_3$), 7.34 (m, 1H, pyrrole H3), 7.65 (m, 1H, pyrrole H5), 9.86 (s, 1H, C*H*O), 10.94 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 61.0, 114.1, 125.0, 127.2, 129.2, 161.1, 185.9.

**5-Formyl-1*H*-pyrrole-2-carboxylic acid 5.46**

A solution of **5.44** (370 mg, 2.21 mmol) and potassium hydroxide (497 mg, 8.85 mmol, 4 equiv) in distilled water (10 mL) was stirred in a warm water bath (40 – 50 °C bath temperature) for 2 h. The solution was then acidified with 10 M aqueous hydrochloric acid and extracted with ether (3x20 mL). The combined ethereal solutions were washed with water (2x10 mL), dried and evaporated under reduced pressure to give **5.46** (251 mg, 82%) as an orange solid: mp >150 °C decomp. (lit.[32] 202 – 203 °C); IR ($CHCl_3$) 3695, 3431, 1709, 1674 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 300 MHz) δ 6.81 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 6.90 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 9.63 (s, 1H, C*H*O), 11.52 (bs, 1H, N*H*); $^{13}C$ NMR (acetone-$d_6$, 75 MHz) δ 114.0, 116.4, 127.2, 134.1, 159.3, 179.0; HRMS calcd for $C_6H_5NO_3$ 139.0269, found 139.0270.

**_N_-(5-Formyl-1_H_-pyrrole-2-carbonyl)glycine ethyl ester 5.47a**

The pyrrole acid **5.46** (153 mg, 1.10 mmol) was coupled with glycine ethyl ester hydrochloride (169 mg, 1.21 mmol, 1.1 equiv) according to general procedure L. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **5.47a** (173 mg, 70%) as a white solid: mp 149 °C, IR ($CHCl_3$) 3425, 1740, 1678, 1553, 1533 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, $J$ = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 4.24 – 4.31 (m, 4H, $CH_2CO_2CH_2CH_3$), 6.73 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 6.93 – 6.96 (m, 2H, pyrrole H4 & CONH), 9.64 (s, 1H, CHO), 10.56 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 41.5, 61.9, 111.1, 119.9, 131.0, 134.0, 160.0, 169.9, 180.3; HRMS calcd for $C_{10}H_{12}N_2O_4$ 224.0797, found 224.0802.

**_N_-(5-Formyl-1_H_-pyrrole-2-carbonyl)-_L_-leucine methyl ester 5.47b**

The pyrrole acid **5.46** (180 mg, 1.29 mmol) was coupled with _L_-leucine methyl ester hydrochloride (259 mg, 1.42 mmol, 1.1 equiv) according to general procedure L. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.47b** (310 mg, 90%) as a pale yellow oil: IR ($CHCl_3$) 3425, 1740, 1676, 1551, 1529 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.94 (m, 6H, $CHMe_2$), 1.59 – 1.73 (m, 3H, $CH_2CHMe_2$), 3.77 (s, 3H, $CO_2Me$), 4.85 (m, 1H, αH), 6.70 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 6.90 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 7.03 (d, $J$ = 7.8 Hz, 1H, CONH), 9.59 (s, 1H, CHO), 10.49 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.8, 22.8, 24.9, 41.5, 50.9, 52.6, 111.3, 120.0, 131.3, 133.9, 159.7, 173.9, 180.2; HRMS calcd for $C_{13}H_{18}N_2O_4$ 266.1267, found 266.1265; Anal. Calcd for $C_{13}H_{18}N_2O_4$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.47; H, 7.14; N, 10.13.

The pyrrole acid **5.46** (311 mg, 2.24 mmol) was also coupled with _L_-leucine methyl ester hydrochloride (406 mg, 2.24 mmol, 1 equiv) according to general procedure K. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **5.47b** (531 mg, 89%) as a yellow oil. Spectral data as recorded above.

**_N_-(5-Formyl-1_H_-pyrrole-2-carbonyl)-_L_-phenylalanine methyl ester 5.47c**

The pyrrole acid **5.46** (210 mg, 1.51 mmol) was coupled with _L_-phenylalanine methyl ester hydrochloride (358 mg, 1.66 mmol, 1.1 equiv) according to general procedure L. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave

**5.47c** (413 mg, 91%) as a pale yellow oil which solidified at 0 °C. An analytical sample was obtained by recrystallisation from petroleum ether to give a white solid: mp 49 °C, IR ($CHCl_3$) 3423, 1742, 1678, 1551, 1529 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.23 (m, 2H, $CH_2Ph$), 3.77 (s, 3H, $CO_2Me$), 5.15 (m, 1H, αH), 6.66 (m, 1H, pyrrole H3), 6.92 (m, 1H, pyrrole H4), 6.99 (d, $J$ = 7.3 Hz, 1H, CONH), 7.11 – 7.31 (m, 5H, phenyl Hs), 9.56 (s, 1H, CHO), 10.82 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 37.9, 52.5, 53.3, 111.4, 120.0, 127.2, 128.6, 129.2, 131.3, 134.0, 135.6, 159.4, 171.9, 180.3; HRMS calcd for $C_{16}H_{16}N_2O_4$ 300.1110, found 300.1109.

## 8.5.3. α-Chymotrypsin assay and results

### 8.5.3.1. General Assay Conditions

The assay was based on a colorimetric technique and carried out in microtitre plates (NUNC flat bottomed plates, GIBCO). The enzyme and test solution were preincubated to ensure maximum inhibition before addition of substrate. After the addition of substrate the optical density was measured over approximately 3 h. The lack of colour development, caused by enzymic release of 4-nitroanilide derivatives, indicated inhibition. Control samples were included in all cases. Each sample was assayed in triplicate. Methanol was used to dissolve the proposed inhibitor and for sample dilution, as it is water soluble and directly compatible with the assay system.

### 8.5.3.2. α-Chymotrypsin Assay

Solutions of the pyrroles **4.8a**, **2.25b**, **5.3a-d**, **5.6a-b**, **5.8a-b**, **5.9a-b** and **5.10b** were made to 1250 μg $mL^{-1}$, 125 μg $mL^{-1}$ and 12.5 μg $mL^{-1}$ in methanol. Tris-HCl (50 μL of 0.4 M solution in water, pH 7.6), distilled water (50 μL), test solution (50 μL) and α-chymotrypsin (50 μL, Sigma ex-Bovine pancreas, 9 units $mL^{-1}$ in 50 mM Tris-HCl buffer, pH 7.6) were added to each well of the microtitre plate. Incubation at 37 °C for 30 min was followed by the addition of *N*-succinyl-*L*-phenylalanine-4-nitroanilide (100

μL, 1 mg $mL^{-1}$ solution in 50 mM Tris-HCl buffer, pH 7.6). The absorbance was read at 405 nm at t = 0, and after incubation at 37 °C when significant colour change had taken place. Each sample was assayed in triplicate and average absorbances were used to calculate the percentage (%) inhibition (see Table 8.1). Sample blanks in which 50 mM Tris-HCl buffer, pH 7.6, replaced α-chymotrypsin were run concurrently.

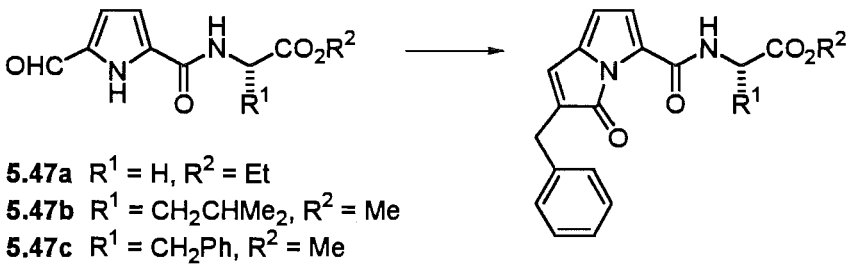
| Compound | % Inhibition | | |
|---|---|---|---|
| | 1250 μg $mL^{-1}$ | 125 μg $mL^{-1}$ | 12.5 μg $mL^{-1}$ |
| **4.8a** | 40 | 10 | 0 |
| **5.3a** | 25 | 10 | 10 |
| **2.25b** | 0 | 0 | 0 |
| **5.3b** | 35 | 20 | 5 |
| **5.3c** | 30 | 15 | 5 |
| **5.3d** | 20 | 10 | 5 |
| **5.6a** | 5 | 5 | 0 |
| **5.6b** | 0* | 0* | 0 |
| **5.8a** | 10 | 5 | 0 |
| **5.8b** | 25 | 10 | 0 |
| **5.9a** | 0 | 0 | 0 |
| **5.9b** | 20 | 15 | 10 |
| **5.10b** | 10 | 5 | 0 |

* precipitation occurred in reaction wells during assay

**Table 8.1.** Results of α-chymotrypsin assay (results rounded to the nearest 5%).

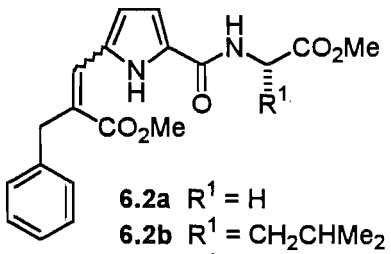
# 8.6 Experimental Work Described in Chapter Six

## 8.6.1. Attempted *N*-acylation of 5.47a-c using general procedure C: Pyrrolizin-3-one formation



**5.47a** $R^1$ = H, $R^2$ = Et
**5.47b** $R^1$ = $CH_2CHMe_2$, $R^2$ = Me
**5.47c** $R^1$ = $CH_2Ph$, $R^2$ = Me

**6.1a** $R^1$ = H, $R^2$ = Et
**6.1b** $R^1$ = $CH_2CHMe_2$, $R^2$ = Me
**6.1c** $R^1$ = $CH_2Ph$, $R^2$ = Me



**6.2a** $R^1$ = H
**6.2b** $R^1$ = $CH_2CHMe_2$
**6.3c** $R^1$ = $CH_2Ph$

***N*-(2-Benzyl-3-oxopyrrolizin-5-ylcarbonyl)glycine ethyl ester 6.1a**

The pyrrole **5.47a** (45 mg, 0.20 mmol) was treated with hydrocinnamoyl chloride (62 μL, 0.42 mmol, 2.1 equiv) according to general procedure C. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **6.1a** (39 mg, 58%) as an orange solid. An analytical sample was recrystallised from ethyl acetate/petroleum ether to give thin orange crystals: mp 104 – 105 °C; UV (MeOH) $\lambda_{max}$ 242 nm (ε 11300), 338 (25400), 423 (200); IR ($CHCl_3$) 3294, 1726, 1647, 1582, 1560 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.30 (t, *J* = 7.3 Hz, 3H, $CO_2CH_2CH_3$), 3.62 (d, *J* = 1.5 Hz, 2H, $CH_2Ph$), 4.19 – 4.28 (m, 4H, $CH_2CO_2CH_2CH_3$), 5.97 (d, *J* = 3.4 Hz, 1H, pyrrolizin-3-one H7), 6.65 (t, *J* = 1.5 Hz, 1H, pyrrolizin-3-one H1), 6.88 (d, *J* = 3.4 Hz, 1H, pyrrolizin-3-one H6), 7.23 – 7.37 (m, 5H, phenyl Hs), 9.33 (t, *J* = 5.4 Hz, 1H, CONH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 31.9, 41.7, 61.4, 111.0, 124.3, 126.9, 128.8 (2xC), 130.3, 132.3, 136.8, 137.0, 139.7,

158.2, 167.8, 169.6; HRMS calcd for $C_{19}H_{18}N_2O_4$ 338.1267, found 338.1264. Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.45; H, 5.36; N, 8.28. Found: C, 67.48; H, 5.32; N, 8.47.

**Crystallographic structure determination for compound 6.1a by X-ray analysis**

$C_{19}H_{18}N_2O_4$, M 338.36, mp 104 – 105 °C, crystal dimensions 0.9 x 0.2 x 0.1 mm, monoclinic, *a* 9.530(16) Å, *b* 10.48(3) Å, *c* 33.36(9) Å, $\beta$ 97.24(6)°, $V$ = 3304(13) Å$^3$, space group $P2_1/c$, $Z$ = 4, F(000) = 1424, $D_{calc}$ = 1.360 mg/m$^3$, absorption coefficient 0.097 mm$^{-1}$, $\theta$ range for data collection 2.30 – 26.54, index ranges $-11 \le h \le 4$, $-13 \le k \le 13$, $-41 \le l \le 41$, data/restraints/ parameters 6758/0/453, goodness of fit on $F^2$ was 0.791, final $R$ indices $[I>2\sigma(I)]$ $R_1$ = 0.0375, $wR_2$ = 0.0716, $R$ indices (all data) $R_1$ = 0.1065, $wR_2$ = 0.0799, largest difference peak and hole 0.211 and –0.220 eÅ$^{-3}$.

A unique data set was measured at 158(2) K within $2\theta_{max}$ = 53° limit ($\omega$ scans). Of the 23851 reflections obtained, 6758 were unique ($R_{int}$ = 0.0538) and were used in the full-matrix least-squares refinement.[17] The structure was solved by direct methods.[18] Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.[19]

***N*-(2-Benzyl-3-oxopyrrolizin-5-ylcarbonyl)-*L*-leucine methyl ester 6.1b**

The pyrrole **5.47b** (50 mg, 0.19 mmol) was treated with hydrocinnamoyl chloride (59 μL, 0.39 mmol, 2.1 equiv) according to general procedure C. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **6.1b** (39 mg, 55%) as an orange oil: UV (MeOH) $\lambda_{max}$ 223 nm (ε 11500), 339 (9100), 436 (900); IR ($CHCl_3$) 3277, 1726, 1645, 1582, 1560 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.94 (d, $J$ = 5.4 Hz, 3H, CH*Me*$_2$), 0.97 (d, $J$ = 5.4 Hz, 3H, CH*Me*$_2$), 1.72 – 1.79 (m, 3H, C*H*$_2$C*H*Me$_2$), 3.62 (d, $J$ = 1.7 Hz, 2H, C*H*$_2$Ph), 3.75 (s, 3H, CO$_2$*Me*), 4.71 (m, 1H, α*H*), 5.96 (d, $J$ = 3.4 Hz, 1H, pyrrolizin-3-one H7), 6.61 (t, $J$ = 1.7 Hz, 1H, pyrrolizin-3-one H1), 6.87 (d, $J$ = 3.4 Hz, 1H, pyrrolizin-3-one H6), 7.24 – 7.37 (m, 5H, phenyl Hs), 9.22 (d, $J$ = 6.8 Hz, 1H, CON*H*); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 21.8, 22.8, 24.9, 31.8, 41.0, 51.3, 52.2, 111.0, 124.3, 126.9, 128.8, 128.9, 130.5, 132.3, 136.9, 137.0, 139.7, 157.9, 167.9, 173.2; HRMS calcd for $C_{22}H_{24}N_2O_4$ 380.1736, found 380.1734.

**_N_-(2-Benzyl-3-oxopyrrolizin-5-ylcarbonyl)-_L_-phenylalanine methyl ester 6.1c**

The pyrrole **5.47c** (59 mg, 0.20 mmol) was treated with hydrocinnamoyl chloride (62 μL, 0.41 mmol, 2.1 equiv) according to general procedure C. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) followed by recrystallisation from ethyl acetate/petroleum ether gave **6.1c** (47 mg, 58%) as orange/red crystals: mp 158 °C; UV ($CHCl_3$) $\lambda_{max}$ 301 nm (ε 12300), 438 (4100); IR ($CHCl_3$) 3516, 3269, 1728, 1647, 1605, 1580, 1558 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.19 (m, 2H, C*H*$_2$Ph), 3.60 (d, *J* = 1.5 Hz, 2H, C*H*$_2$Ph), 3.72 (s, 3H, $CO_2$*Me*), 4.96 (m, 1H, α*H*), 5.94 (d, *J* = 3.4 Hz, 1H, pyrrolizin-3-one H7), 6.60 (t, *J* = 1.5 Hz, 1H, pyrrolizin-3-one H1), 6.84 (d, *J* = 3.4 Hz, 1H, pyrrolizin-3-one H6), 7.21 – 7.38 (m, 10H, phenyl Hs), 9.31 (t, *J* = 7.3 Hz, 1H, CON*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 31.9, 38.0, 52.3, 54.3, 110.9, 124.3, 126.9 (2xC), 128.4, 128.8, 128.9, 129.2, 130.3, 132.2, 136.3, 136.9, 137.1, 139.7, 157.8, 167.6, 172.0; HRMS calcd for $C_{25}H_{22}N_2O_4$ 414.1580, found 414.1592. Anal. Calcd for $C_{25}H_{22}N_2O_4$: C, 72.45; H, 5.35; N, 6.76. Found: C, 72.68; H, 5.47; N, 6.95.

**_N_-[5-(2-Carbomethoxy-3-phenyl-1-propenyl)-1_H_-pyrrole-2-carbonyl]glycine methyl ester 6.2a**

To a stirred solution of the pyrrolizin-3-one **6.1a** (12 mg, 0.04 mmol) in methanol (3 mL) at rt under $N_2$ was added sodium methoxide (29 mg, 0.53 mmol, 15 equiv) dissolved in methanol (2 mL). After stirring for 1 min the solution was acidified with 1 M aqueous sulfuric acid and extracted with dichloromethane (2x10 mL). The combined organic fractions were washed with water (2x10 mL), saturated aqueous brine (10 mL), dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) gave **6.2a** (11 mg, 85%) as a mixture of the *cis*- and *trans*-isomers (**3 : 1** by $^1H$ NMR) as a white solid: $^1H$ NMR ($CDCl_3$, 300 MHz) of *cis*-isomer assigned from mixture, δ 3.67 (s, 5H, $CO_2$*Me* & C*H*$_2$Ph), 3.71 (s, 3H, $CO_2$*Me*), 4.15 (d, *J* = 5.4 Hz, 2H, C*H*$_2CO_2$), 6.29 (dd, *J* = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.43 (t, *J* = 5.4 Hz, 1H, CON*H*), 6.54 (dd, *J* = 3.9 & 2.4 Hz, 1H, pyrrole H3), 6.59 (s, 1H, acrylic H), 7.11 – 7.24 (m, 5H, phenyl Hs), 12.38 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) of *cis*-isomer assigned from mixture, δ 33.6, 41.2, 52.2, 52.4, 110.2, 118.1, 124.2, 126.3, 128.4, 128.6, 129.9, 130.7, 131.9, 139.6, 160.5, 168.6, 170.6. This sample was recrystallised from ethyl acetate/petroleum ether to give the *trans*-isomer as a white crystalline solid (3 mg,

22%): mp 91 – 92 °C; IR ($CHCl_3$) 3435, 3306, 1746, 1697, 1655, 1605, 1541, 1516 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) of *trans*-isomer, δ 3.77 (s, 3H, $CO_2Me$), 3.78 (s, 3H, $CO_2Me$), 4.03 (s, 2H, $CH_2Ph$), 4.26 (d, $J$ = 4.9 Hz, 2H, $CH_2CO_2$), 6.43 - 6.47 (m, 2H, pyrrole H4 & CONH), 6.61 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 7.19 – 7.31 (m, 5H, phenyl Hs), 7.83 (s, 1H, acrylic H), 10.21 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) of *trans*-isomer, δ 33.6, 41.2, 52.2, 52.5, 111.4, 114.1, 126.4, 127.2, 127.5, 127.9, 128.7, 129.8, 131.3, 138.3, 160.4, 168.6, 170.4; HRMS calcd for $C_{19}H_{20}N_2O_5$ 356.1372, found 356.1379.

**N-{(Z)-5-[2-Carbomethoxy-3-phenyl-1-propenyl]-1H-pyrrole-2-carbonyl}-L-leucine methyl ester 6.2b**

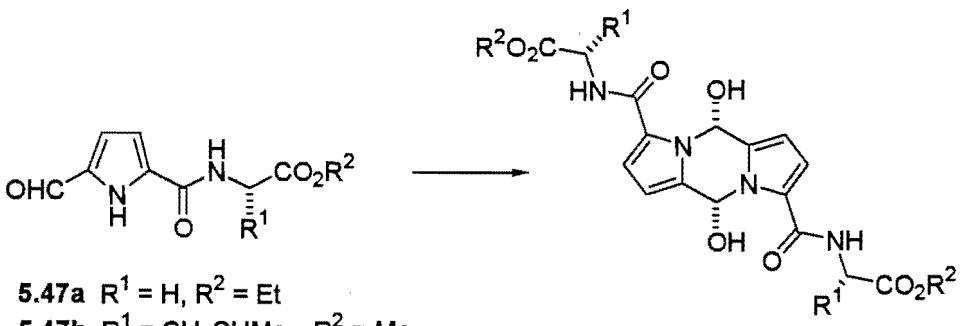
The pyrrolizin-3-one **6.1b** (25 mg, 0.07 mmol) was treated with sodium methoxide (54 mg, 0.99 mmol, 15 equiv) by the method described above for the preparation of **6.2a**. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) gave **6.2b** (22 mg, 80%) as a white solid. An analytical sample was recrystallised from ethyl acetate/petroleum ether to give white crystals: mp 113 – 114 °C; IR ($CHCl_3$) 3439, 3310, 1738, 1699, 1655, 1514 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.96 (m, 6H, $CHMe_2$), 1.61 – 1.75 (m, 3H, $CH_2CHMe_2$), 3.74 (s, 5H, $CO_2Me$ & $CH_2Ph$), 3.75 (s, 3H, $CO_2Me$), 4.85 (m, 1H, αH), 6.36 (dd, $J$ = 3.7 & 2.4 Hz, 1H, pyrrole H4), 6.40 (d, $J$ = 8.5 Hz, 1H, CONH), 6.60 (dd, $J$ = 3.7 & 2.4 Hz, 1H, pyrrole H3), 6.65 (s, 1H, acrylic H), 7.18 – 7.31 (m, 5H, phenyl Hs), 12.43 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 22.0, 22.8, 24.9, 41.3, 42.0, 50.6, 52.2, 52.3, 109.9, 118.1, 124.0, 126.3, 127.7, 128.4, 128.6, 130.7, 132.0, 136.9, 139.6, 142.7, 160.1, 168.5, 173.7; HRMS calcd for $C_{23}H_{28}N_2O_5$ 412.1998, found 412.2001. Anal. Calcd for $C_{23}H_{28}N_2O_5$: C, 66.97; H, 6.84; N, 6.79. Found: C, 67.07; H, 6.55; N, 6.61.

**N-{(Z)-5-[2-Carbomethoxy-3-phenyl-1-propenyl]-1H-pyrrole-2-carbonyl}-L-phenylalanine methyl ester 6.2c**

The pyrrolizin-3-one **6.1c** (16 mg, 0.04 mmol) was treated with sodium methoxide (31 mg, 0.58 mmol, 15 equiv) by the method described above for the preparation of **6.2a**. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **6.2c** (14 mg, 82%) as a white solid: mp 43 – 44 °C; IR ($CHCl_3$) 3429, 3296, 1742,

1697, 1655, 1607, 1541, 1514 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 3.23 (m, 2H, $CH_2Ph$), 3.74 – 3.76 (m, 8H, $2xCO_2Me$ & $CH_2Ph$), 5.06 (m, 1H, αH), 6.34 – 6.36 (m, 2H, pyrrole H4 & CONH), 6.50 (m, 1H, pyrrole H3), 6.67 (s, 1H, acrylic H), 7.11 – 7.31 (m, 10H, phenyl Hs), 12.48 (bs, 1H, NH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 38.1, 41.2, 52.2, 52.3, 53.1, 110.1, 118.1, 124.1, 126.3, 127.1, 127.6, 128.4, 128.6 (2xC), 129.3, 130.7, 132.0, 135.8, 139.6, 159.8, 168.6, 172.0; HRMS calcd for $C_{26}H_{26}N_2O_5$ 446.1842, found 446.1849.

## 8.6.2. Attempted *N*-acylation of 5.47a-c using general procedure B: Azafulvene dimer formation



**5.47a** $R^1$ = H, $R^2$ = Et
**5.47b** $R^1$ = $CH_2CHMe_2$, $R^2$ = Me
**5.47c** $R^1$ = $CH_2Ph$, $R^2$ = Me

**6.6a** $R^1$ = H, $R^2$ = Et
**6.6b** $R^1$ = $CH_2CHMe_2$, $R^2$ = Me
**6.6c** $R^1$ = $CH_2Ph$, $R^2$ = Me

**Glycine-based azafulvene dimer 6.6a**

The modified general procedure B was carried out using pyrrole **5.47a** (48 mg, 0.22 mmol) and acetyl chloride (15 μL, 0.22 mmol, 1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 2:1) followed by recrystallisation from ethyl acetate/petroleum ether gave **6.6a** (24 mg, 50%) as colourless crystals: mp 137 – 139 °C; IR ($CHCl_3$) 3350, 1732, 1638, 1560, 1535 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 1.36 (t, $J$ = 7.3 Hz, 6H, $CO_2CH_2CH_3$), 3.69 (dd, $J$ = 18.1 & 4.4 Hz, 2H, $CH_2CO_2$), 4.34 (q, $J$ = 7.3 Hz, 4H, $CO_2CH_2CH_3$), 4.69 (dd, $J$ = 18.1 & 8.3 Hz, 2H, $CH_2CO_2$), 6.27 (d, $J$ = 3.9 Hz, 2H, H-1,6), 6.29 (d, $J$ = 3.9 Hz, 2H, H-5,10), 6.50 (d, $J$ = 3.9 Hz, 2H, OH), 6.90 (d, $J$ = 3.9 Hz, 2H, H-2,7), 8.52 (m, 2H, CONH); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 41.0, 62.3,

71.6, 108.0, 115.1, 124.3, 130.8, 162.1, 173.0; HRMS (FAB) calcd for $C_{20}H_{23}N_4O_7$ (MH-$H_2O$) 431.1567, found 431.1564.

Upon standing for 3 days in $CDCl_3$ in a 3 mm NMR tube, approximately 15% of a second compound was observed to form. Selected $^1H$ NMR ($CDCl_3$, 300 MHz) of second compound assigned from the mixture, δ 6.35 (d, $J$ = 3.9 Hz, 2H), 6.61 (d, $J$ = 3.9 Hz, 2H), 6.93 (d, $J$ = 3.9 Hz, 2H), 7.06 (d, $J$ = 3.9 Hz, 2H), 7.69 (m, 2H).

**_L_-Leucine-based azafulvene dimer 6.6b**

The modified general procedure B was carried out using pyrrole **5.47b** (47 mg, 0.18 mmol) and acetyl chloride (13 μL, 0.18 mmol, 1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) followed by recrystallisation from ethyl acetate/petroleum ether gave **6.6b** (19 mg, 41%) as colourless crystals: mp 166 °C; $[\alpha]_D^{25} = -120.6°$ (c = 0.165, $CH_2Cl_2$); IR ($CHCl_3$) 3342, 1732, 1632, 1553 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, $J$ = 6.3 Hz, 6H, CH*Me*$_2$), 0.96 (d, $J$ = 6.8 Hz, 6H, CH*Me*$_2$), 1.61 – 1.87 (m, 6H, C*H*$_2$C*H*Me$_2$), 3.89 (s, 6H, CO$_2$*Me*), 4.75 (m, 2H, α*H*), 6.21 – 6.23 (m, 4H, H-1,6 & H-5,10), 6.49 (d, $J$ = 3.9 Hz, 2H, O*H*), 6.88 (d, $J$ = 4.4 Hz, 2H, H-2,7), 8.46 (d, $J$ = 7.8 Hz, 2H, CON*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 21.0, 23.2, 25.0, 40.2, 50.9, 52.9, 71.5, 107.9, 115.0, 124.3, 130.7, 161.9, 176.9; HRMS (EI) calcd for $C_{13}H_{18}N_2O_4$ (½M) 266.1267, found 266.1273; HRMS (FAB) calcd for $C_{26}H_{35}N_4O_7$ (MH-$H_2O$) 515.2506, found 515.2519. Anal. Calcd for $C_{26}H_{36}N_4O_8$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.25; H, 6.95; N, 10.21.

The reaction was repeated using pyrrole **5.47b** (48 mg, 0.18 mmol) and hydrocinnamoyl chloride (27 μL, 0.18 mmol, 1 equiv) to give **6.6b** (12 mg, 25%). Spectral data as recorded above.

**Crystallographic structure determination for compound 6.6b by X-ray analysis**

$C_{26}H_{20}N_4O_8.C_4H_8O_2$, M 604.62, mp 166 °C, crystal dimensions 0.73 x 0.4 x 0.4 mm, tetragonal, $a$ 13.1462(4) Å, $b$ 13.1462(4) Å, $c$ 19.5541(8) Å, $V$ = 3379.4(2) Å$^3$, space group $P4_22_12$, $Z$ = 8, F(000) = 1280, $D_{calc}$ = 1.188 mg/m$^3$, absorption coefficient 0.089 mm$^{-1}$, $\theta$ range for data collection 1.87 – 21.96°, index ranges $-13 \le h \le 13$, $-13 \le k \le 13$, $-20 \le l \le 18$, data/restraints/ parameters 2073/8/218, goodness of fit on $F^2$ was 1.077,

final $R$ indices [$I>2\sigma(I)$] $R_1 = 0.0562$, $wR_2 = 0.1335$, $R$ indices (all data) $R_1 = 0.0620$, $wR_2 = 0.1382$, largest difference peak and hole 0.478 and –0.292 eÅ$^{-3}$.

A unique data set was measured at 144(2) K within $2\theta_{max} = 44°$ limit ($\omega$ scans). Of the 11262 reflections obtained, 2073 were unique ($R_{int} = 0.1024$) and were used in the full-matrix least-squares refinement.[17] The structure was solved by direct methods.[18] Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.[19]

Analytical data was consistent with the presence of one ethyl acetate solvent molecule in the asymmetric unit. However, electron density maps showed this molecule to be disordered and positioned across a two fold rotational axis of symmetry. In this situation the best fitting model was necessarily distorted by self superposition, therefore its geometry is poorly defined. Electron density maps also showed a small degree of disorder present in the terminal methyl groups of the leucine side chains. However, all attempts to model this disorder were unsatisfactory.

**_L_-Phenylalanine-based azafulvene dimer 6.6c**

The modified general procedure B was carried out using pyrrole **5.47c** (52 mg, 0.17 mmol) and acetyl chloride (12 μL, 0.17 mmol, 1 equiv). Flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) followed by recrystallisation from ethyl acetate/petroleum ether gave **6.6c** (13 mg, 25%) as colourless crystals: mp 133 – 135 °C; $[\alpha]_D^{25} = -149.0°$ (c = 0.05, $CH_2Cl_2$); IR ($CHCl_3$) 3427, 3352, 1732, 1636, 1551, 1528 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 3.15 (m, 4H, C*H*$_2$Ph), 3.90 (s, 6H, $CO_2$*Me*), 4.98 (m, 2H, α*H*), 6.17 (d, $J$ = 4.4 Hz, 2H, H-1,6), 6.23 (d, $J$ = 3.9 Hz, 2H, H-5,10), 6.27 (d, $J$ = 3.9 Hz, 2H, O*H*), 6.72 (d, $J$ = 4.4 Hz, 2H, H-2,7), 7.21 – 7.30 (m, 10H, phenyl Hs), 8.16 (d, $J$ = 7.3 Hz, 2H, CON*H*); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 37.7, 53.0, 54.0, 71.5, 107.9, 114.8, 124.0, 127.3, 128.7, 128.9, 130.7, 136.1, 161.6, 175.2; HRMS (FAB) calcd for $C_{32}H_{31}N_4O_7$ (MH-$H_2O$) 583.2193, found 583.2183.

**Crystallographic structure determination for compound 6.6c by X-ray analysis**

$C_{26}H_{36}N_4O_8$, M 532.59, mp 133 – 135 °C, crystal dimensions 0.45 x 0.30 x 0.26 mm, monoclinic, *a* 25.748(4) Å, *b* 10.440(5) Å, *c* 24.801(4) Å, $\beta$ 96.548(12)°, $V$ = 6624(3) Å$^3$, space group $P2_1$, $Z$ = 4, F(000) = 2528, $D_{calc}$ = 1.205 mg/m$^3$, absorption coefficient 0.727 mm$^{-1}$, $\theta$ range for data collection 3.46 – 47.23, index ranges $0 \leq h \leq 24$, $0 \leq k \leq 9$, $-23 \leq l \leq 23$, data/restraints/ parameters 3224/137/788, goodness of fit on $F^2$ was 1.223, final $R$ indices [$I>2\sigma(I)$] $R_1$ = 0.0958, $wR_2$ = 0.2344, $R$ indices (all data) $R_1$ = 0.1132, $wR_2$ = 0.2529, largest difference peak and hole 1.059 and –0.367 eÅ$^{-3}$.

A unique data set was measured at 293(2) K within $2\theta_{max}$ = 94° limit ($\omega$ scans). Of the 3320 reflections obtained, 3224 were unique ($R_{int}$ = 0.0447) and were used in the full-matrix least-squares refinement.[17] The structure was solved by direct methods.[18] Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton.

# 8.7 Experimental Work Described in Chapter Seven

## 8.7.1. Preparation of acid chlorides[33]

**_n_-Hexanoyl chloride**

Into a 50 mL RBF fitted with a reflux condenser was placed *n*-hexanoic acid (7.00g, 60.3 mmol) and oxalyl chloride (6.31 mL, 72.3 mmol, 1.2 equiv), and the resultant mixture was heated and stirred at 60 – 70 °C for 2 h. The crude acid chloride was then distilled at reduced pressure (oil pump) to give *n*-hexanoyl chloride (7.60 g, 94%) as a colourless oil: IR ($CHCl_3$) 1794 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.91 (m, 3H, H-6), 1.31 – 1.37 (m, 4H, H-4 & H-5), 1.72 (m, 2H, H-3), 2.89 (t, $J$ = 7.3 Hz, 2H, H-2).

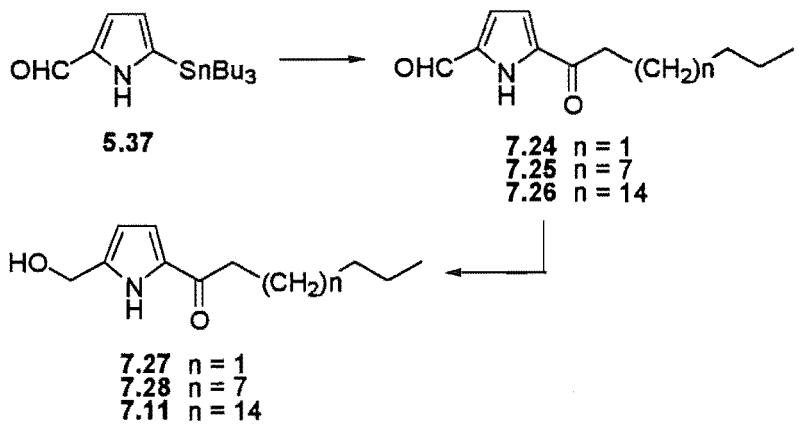
**Dodecanoyl (lauroyl) chloride**

Lauroyl chloride (8.32 g, 95%) was prepared from lauric acid (8.00 g, 39.9 mmol) and oxalyl chloride (4.18 mL, 47.9 mmol, 1.2 equiv) by the method described above for *n*-hexanoyl chloride. IR ($CHCl_3$) 1794 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, $J$ = 6.8 Hz, 3H, H-12), 1.27 – 1.31 (broad signal, 16H, H-4 – H-11), 1.71 (m, 2H, H-3), 2.88 (t, $J$ = 7.3 Hz, 2H, H-2).

**Nonadecanoyl chloride**

Nonadecanoic acid (200 mg, 0.67 mmol) was dissolved in benzene (5 mL) and oxalyl chloride (70 μL, 0.80 mmol, 1.2 equiv) was added. The stirred mixture was heated at 60 –70 °C for 2 h. The solvent was then removed at reduced pressure to give the crude acid chloride as a white solid (208 mg, 98%) which was kept under high vacuum (oil pump) until required: IR ($CHCl_3$) 1794 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, $J$ = 6.8 Hz, 3H, H-17), 1.26 – 1.31 (broad signal, 30H, H-4 – H-18), 1.71 (m, 2H, H-3), 2.88 (t, $J$ = 7.3 Hz, 2H, H-2).

## 8.7.2. Preparation of hydroxymethylpyrroles 7.27, 7.28 and 7.11




**5-(1-Oxohexyl)-1*H*-pyrrole-2-carboxaldehyde 7.24**

The stannylpyrrole **5.37** (150 mg, 0.39 mmol) was coupled with *n*-hexanoyl chloride (55 μL, 0.39 mmol, 1 equiv) according to general procedure M. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:5) gave **7.24** (58 mg, 77%) as a white solid: mp 48 °C (lit.[34] 48 – 49 °C); IR ($CHCl_3$) 3422, 1678, 1659 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.91 (m, 3H, H-11), 1.32 – 1.38 (m, 4H, H-9 & H-10), 1.74 (m, 2H, H-8), 2.85 (t, $J$ = 7.3 Hz, 2H, H-7), 6.91 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.97 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 9.73 (s, 1H, C*H*O), 10.25 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 13.8, 22.4, 24.1, 31.4, 38.7, 115.3, 119.5, 134.7, 135.5, 180.9, 192.3; HRMS calcd for $C_{11}H_{15}NO_2$ 193.1103, found 193.1104.

**5-(1-Oxododecyl)-1*H*-pyrrole-2-carboxaldehyde 7.25**

The stannylpyrrole **5.37** (150 mg, 0.39 mmol) was coupled with lauroyl chloride (90 μL, 0.39 mmol, 1 equiv) according to general procedure M. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:6) gave **7.25** (99 mg, 91%) as a white solid. An analytical sample was obtained by recrystallisation from petroleum ether to give a fine white solid: mp 66 °C; IR ($CHCl_3$) 3422, 1678, 1661 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, $J$ = 6.8 Hz, 3H, H-17), 1.26 – 1.33 (broad signal, 16H, H-9 – H-16), 1.73 (m, 2H, H-8), 2.84 (t, $J$ = 7.3 Hz, 2H, H-7), 6.90 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.96 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 9.72 (s, 1H, C*H*O), 10.06 (bs, 1H, N*H*); $^{13}C$ NMR

($CDCl_3$, 75 MHz) δ 14.1, 22.6, 24.5, 29.3 – 29.6 (6xC), 31.9, 38.8, 115.2, 119.5, 134.7, 135.5, 180.8, 192.3; HRMS calcd for $C_{17}H_{27}NO_2$ 277.2042, found 277.2034.

**5-(1-Oxononadecyl)-1*H*-pyrrole-2-carboxaldehyde 7.26**

The stannylpyrrole **5.37** (150 mg, 0.39 mmol) was coupled with nonadecanoyl chloride (124 mg, 0.39 mmol, 1 equiv) according to general procedure M. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:8) gave **7.26** (135 mg, 92%) as a white solid. An analytical sample was obtained by recrystallisation from petroleum ether to give a fine white solid: mp 85 °C; IR ($CHCl_3$) 3422, 1678, 1659 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, $J$ = 6.8 Hz, 3H, H-24), 1.25 – 1.32 (broad signal, 30H, H-9 – H-23), 1.73 (m, 2H, H-8), 2.83 (t, $J$ = 7.6 Hz, 2H, H-7), 6.90 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.96 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 9.72 (s, 1H, C*H*O), 10.01 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.7, 24.5, 29.3 – 29.7 (13xC), 31.9, 38.8, 115.2, 119.5, 134.6, 135.5, 180.8, 192.3; HRMS calcd for $C_{24}H_{41}NO_2$ 375.3137, found 375.3131.

**2-Hydroxymethyl-5-(1-oxohexyl)-1*H*-pyrrole 7.27**

The pyrrole **7.24** (40 mg, 0.21 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **7.27** (47 mg, 93%) as a pale pink solid: mp 55 – 56 °C, IR ($CHCl_3$) 3439, 3308, 1620 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.90 (m, 3H, H-11), 1.32 – 1.38 (m, 4H, H-9 & H-10), 1.72 (m, 2H, H-8), 2.78 (t, $J$ = 7.3 Hz, 2H, H-7), 4.27 (bs, 1H, OH), 4.74 (s, 2H, C*H*$_2$OH), 6.15 (dd, $J$ = 3.4 & 2.4 Hz, 1H, pyrrole H3), 6.94 (dd, $J$ = 3.4 & 2.4 Hz, 1H, pyrrole H4), 11.12 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 13.9, 22.4, 25.3, 31.5, 37.7, 57.7, 108.8, 118.5, 131.5, 140.9, 192.4; HRMS calcd for $C_{11}H_{17}NO_2$ 195.1259, found 195.1261.

**2-Hydroxymethyl-5-(1-oxododecyl)-1*H*-pyrrole 7.28**

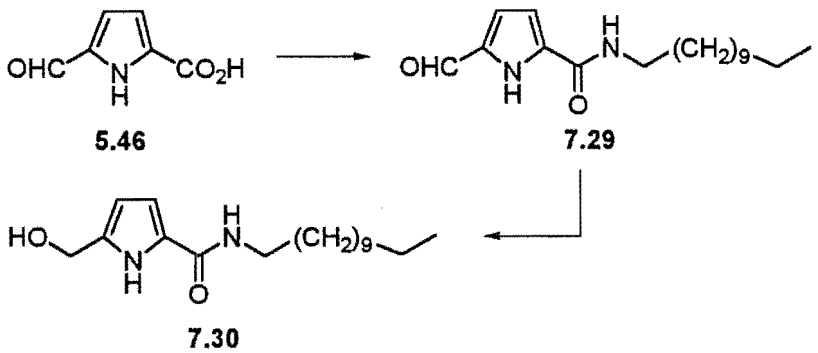
The pyrrole **7.25** (30 mg, 0.11 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **7.28** (25 mg, 84%) as a white solid: mp 70 – 71 °C; IR ($CHCl_3$) 3439, 3308, 1618 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, $J$ = 6.8 Hz, 3H, H-17), 1.25 – 1.32 (broad

signal, 16H, H-9 – H-16), 1.71 (m, 2H, H-8), 2.78 (t, $J$ = 7.6 Hz, 2H, H-7), 4.26 (bs, 1H, OH), 4.73 (s, 2H, C$H_2$OH), 6.15 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 6.93 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 11.11 (bs, 1H, N$H$); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.6, 25.7, 29.3 – 29.6 (6xC), 31.9, 37.8, 57.7, 108.8, 118.5, 131.5, 140.9, 192.4; HRMS calcd for $C_{17}H_{29}NO_2$ 279.2198, found 279.2197.

**2-Hydroxymethyl-5-(1-oxononadecyl)-1*H*-pyrrole 7.11 (Mycalazol 11)**

The pyrrole **7.26** (35 mg, 0.09 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **7.11** (33 mg, 93%) as a fine white solid. An analytical sample was obtained by recrystallisation from methanol to give fine white crystals: mp 78 °C (lit.[35] amorphous powder); IR ($CHCl_3$) 3439, 3306, 1624 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, $J$ = 6.8 Hz, 3H, H-24), 1.25 – 1.32 (broad signal, 30H, H-9 – H-23), 1.72 (m, 2H, H-8), 2.78 (t, $J$ = 7.6 Hz, 2H, H-7), 4.13 (bs, 1H, OH), 4.74 (s, 2H, C$H_2$OH), 6.15 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.93 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 11.04 (bs, 1H, N$H$); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 14.1, 22.7, 25.7, 29.3 – 29.7 (13xC), 31.9, 37.8, 57.8, 108.8, 118.4, 131.5, 140.8, 192.4; HRMS calcd for $C_{24}H_{43}NO_2$ 377.3294, found 377.3296.

## 8.7.3. Preparation of hydroxymethylpyrrole 7.30



**5-Dodecylcarboxamido-1*H*-pyrrole-2-carboxaldehyde 7.29**

To a stirred solution (~0.1 M) of the pyrrole acid **5.46** (100 mg, 0.72 mmol) and dodecylamine (147 mg, 0.79 mmol, 1.1 equiv) in dichloromethane (8 mL) under $N_2$ at rt were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (207 mg, 1.08 mmol, 1.5 equiv) and 1-hydroxybenzotriazole hydrate (194 mg, 1.44 mmol, 2.0 equiv). The reaction mixture was stirred at rt for 16 h, then diluted with dichloromethane (10 mL), washed with 3 M aqueous hydrochloric acid (2x10 mL), water (2x10 mL), saturated aqueous brine (10 mL) dried and evaporated under reduced pressure. Flash chromatography on silica (ethyl acetate/petroleum ether, 1:2) gave **7.29** (202 mg, 92%) as a white solid: mp 79 °C, IR ($CHCl_3$) 3422, 1676, 1553, 1535 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, $J$ = 6.8 Hz, 3H, H-19), 1.25 – 1.32 (broad signal, 18H, H-10 – H-18), 1.60 (m, 2H, H-9), 3.45 (q, $J$ = 6.8 Hz, 2H, H-8), 6.55 (t, $J$ = 5.4 Hz, 1H, CON*H*), 6.68 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H4), 6.96 (dd, $J$ = 3.9 & 2.4 Hz, 1H, pyrrole H3), 9.63 (s, 1H, C*H*O), 10.78 (bs, 1H, N*H*); $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ 14.0, 22.6, 26.9, 29.3 – 29.6 (7xC), 31.8, 39.9, 111.3, 120.1, 132.4, 133.8, 160.1, 180.4; HRMS calcd for $C_{18}H_{30}N_2O_2$ 306.2307, found 306.2311.

**2-Hydroxymethyl-5-dodecylcarboxamido-1*H*-pyrrole 7.30**

The pyrrole **7.29** (50 mg, 0.16 mmol) was reduced with zinc borohydride by general procedure D. Flash chromatography on silica (ethyl acetate/petroleum ether, 2:1) gave **7.30** (46 mg, 91%) as a white solid. An analytical sample was obtained by recrystallisation from methanol to give a white solid: mp 127 °C, IR ($CHCl_3$) 3445, 3304, 1628, 1537 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 300 MHz) δ 0.85 (t, $J$ = 6.6 Hz, 3H, H-19), 1.23 (broad signal, 18H, H-10 – H-18), 1.46 (m, 2H, H-9), 3.17 (q, $J$ = 6.8 Hz, 2H, H-8), 4.38 (d, $J$ = 5.6 Hz, 2H, C$H_2$OH), 4.90 (t, $J$ = 5.6 Hz, 1H, O*H*), 5.94 (d, $J$ = 3.9 Hz, 1H, pyrrole H3), 6.63 (d, $J$ = 3.9 Hz, 1H, pyrrole H4), 7.87 (t, $J$ = 5.6 Hz, 1H, CON*H*), 11.10 (bs, 1H, N*H*); $^{13}C$ NMR (DMSO-$d_6$, 75 MHz) δ 14.0, 22.2, 26.6, 28.8 – 29.2 (6xC), 29.6, 31.4, 38.5, 56.1 106.8, 110.0, 125.8, 136.2, 160.6; HRMS calcd for $C_{18}H_{32}N_2O_2$ 308.2464, found 308.2459.

## 8.7.4. P388 Cytotoxicity assay and results

### P388 Cytotoxicity assay

The P388 murine leukemia cell line (ATCC CCL 46, $P388D_1$) was used to assay for cytoxicity, with activities expressed as an $ID_{50}$ in $\mu g\ mL^{-1}$. MTT tetrazolium (yellow colour) is reduced to MTT formazan (purple colour) by healthy cells, so this can be used to determine the concentration of a sample required to reduce the P388 cell growth by 50% relative to the control.[36] Media, solvent, cell and positive controls were included with each assay run. The results of the cytotoxicity assay are presented in Table 8.2.

| **Compound** | **7.27** | **7.28** | **7.11** | **7.30** |
|---|---|---|---|---|
| Solvent* | A | A | A‡ | B |
| $ID_{50}$ ($\mu g\ mL^{-1}$) | 78 | 21 | 24§ | 52 |

* Solvent: A = 3:1 $MeOH/CH_2Cl_2$; B = MeOH

**Table 8.2.** Cytotoxicity data ($ID_{50}$, $\mu g\ mL^{-1}$) of the hydroxymethylpyrroles **7.27**, **7.28**, **7.11** and **7.30**.

‡ Compound also assayed with solvent = 1:1 $MeOH/CH_2Cl_2$, $ID_{50} > 125\ \mu g\ mL^{-1}$.

§ A precipitate was observed to form in the reaction wells of **7.11** during the P388 assay.

# 8.8 References for Experimental


1 Fischer, H.; Orth, H. *Die Chemie des Pyrrols.* Akademische Verlag: Leipzig, 1934, Vol 2.

2 Still, W. C.; Khan, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

3 (a) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals.* Pergamon Press: London, 1986, 2nd Edn. (b) Furniss, B. S.; Hannaford, A. J.; Smith, P. N. G.; Tatchell, A. R. Eds. *Vogel's Textbook of Practical Organic Chemistry.* Longman: London, 1989, 5th Edn.

4 Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165.

5 Silverstein, R.; Ryskiewicz, E. E.; Willard, C. *Org. Synth., Coll. Vol.* **1963**, *4*, 831.

6 Merrill, B. A.; LeGoff, E. *J. Org. Chem.* **1990**, *55*, 2904.

7 Tietze, L. F.; Kettschau, G.; Heitmann, K. *Synthesis* **1996**, 851.

8 Nickisch, K.; Klose, W.; Bohlmann F. *Chem. Ber.* **1980**, *113*, 2036.

9 (a) Schauder, J-R.; Jendrezejewski, S.; Abell, A. D.; Hart, G. J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1987**, 436; (b) Arigoni, D.; Besmer, P. see Besmer, P. Diss. ETH, No 4435, 1970.

10 Loader, C. E.; Anderson H. J. *Synthesis* **1978**, 295.

11 Wallace, D. M.; Leung, S. H.; Senge, M. O.; Smith, K. M. *J. Org. Chem.* **1993**, *58*, 7245.

12 Prinzbach, H.; Bingmann, H.; Fritz, H.; Markert, J.; Knothe, L.; Eberbach, W.; Brokatzky-Geiger, J.; Sekutowski, J. C.; Krüger, C. *Chem. Ber.* **1986**, *119*, 616.

13 Clezy, P. S.; Liepa, A. J.; Webb, N. W. *Aust. J. Chem.* **1972**, *25*, 1991.

14 Abell, A. D.; Nabbs, B. K.; Battersby, A. R. *J. Org. Chem.* **1998**, *63*, 8163.

15 Trepanier, D. L.; Sprancmanis, V.; Wiggs, K. G. *J. Org. Chem.* **1964**, *29*, 668.

16 Bartlett, P. D.; Knox, L. H. *Org. Synth., Coll. Vol.* **1973**, *5*, 194.

17 Sheldrick, G. M. SHELXL 93, *J. Appl. Crystallogr.* in the press.

18 Sheldrick, G. M. *Acta Crystallogr., Sect. A.* **1990**, *A46*, 467.

19 Ibers, J. A.; Hamilton, W. C. Eds. *International Tables for Crystallography.* Kynoch Press: Birmingham, 1992, Vol. C.

20 Kipping, F. S. *J. Chem. Soc.* **1894**, *65*, 480.

21 Abell, A. D.; Litten, J. C. *Aust. J. Chem.* **1993**, *46*, 1473.

22 Hinz, W.; Jones, R. A.; Anderson, T. *Synthesis* **1986**, 620.

23 Evans, E. B.; Mabbott, E. E.; Turner, E. E. *J. Chem. Soc.* **1927**, *130*, 1159.

24 Denney, D. B.; Ross, S. T. *J. Org. Chem.* **1962**, *27*, 998.

25 Jones, R. A.; Lindner, J. A. *Aust. J. Chem.* **1965**, *18*, 875.

26 Martin, W.; Dürckheimer, W. *Tetrahedron Lett.* **1973**, 1459.

27 Cason, J. *Org. Synth., Coll. Vol.* **1955**, *3*, 169.

28 Eraksina, V. N.; Ivanova, T. M.; Babushkina, T. A.; Vasil'ev, A. M.; Suvorov, N. N. *Khim. Geterotsikl. Soedin.* **1979**, *7*, 916.

29 Muchowski, J. M.; Hess, P. *Tetrahedron Lett.* **1988**, *29*, 3215.

30 Bray, B. L.; Muchowski, J. M. *Can. J. Chem.* **1990**, *68*, 1305.

31 Bailey, D. M.; Johnson, R. E.; Albertson, N. F. *Org. Synth.* **1971**, *51*, 100.

32 Khan, M. K. A; Morgan, K. J.; Morrey, D. P. *Tetrahedron* **1966**, *22*, 2095.

33 Bauer, S. T. *Oil and Soap* **1946**, 1.

34 Muchowski, J. M.; Hess, P. *Tetrahedron Lett.* **1988**, *29*, 777.

35 Ortega, M. J.; Zubia, E.; Carballo, L.; Salvá, J, *Tetrahedron* **1997**, *53*, 331.

36 Boyd, M. R. *Principles and Practice of Oncology* **1989**, *3*, 1.